A COURSE

OF

PRACTICAL CHEMISTRY

FOR

AGRICULTURAL STUDENTS

VOLUME II, PART I

BY

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PREFACE

THE following exercises in Practical Agricultural Chemistry are designed primarily for the use of students taking the course for the Degree in Agricultural Science at Cambridge University. Volume I is intended to cover the first year's course on the Chemistry and Physics of the Soil, while Volume II covers the second year's course on the Chemistry of Foods. The latter is divided into two parts: Part I dealing with such exercises in Pure Organic Chemistry as are essential for agricultural students, and is entirely qualitative, while Part II deals with quantitative estimations and technical analyses of food stuffs. This division has been considered desirable, as students who have already taken a course in Organic Chemistry will find it unnecessary to work through Part I of Volume II. Volume III deals with the special advanced Chemistry of Insecticides and Fungicides, Food Preservatives, etc. It is hoped that this division into convenient booklets will make them useful to teachers. The exercises are designed to illustrate most of the essential points in Agricultural Chemistry and to require the minimum of apparatus.

A Course of Practical Work was compiled by Professor T. B. Wood some years ago for the use of his classes, and this forms the foundation of a portion of the present exercises. The sincere thanks of the authors are due to Professor Wood and the Syndics of the University Press for their permission to utilise that material.

In addition to the directions for practical work a series of notes has been added to each day's work to explain just the points which considerable experience in teaching in a laboratory has shown to be those on which the student usually needs assistance from the demonstrator to explain the reason and reactions of the experiments as they are being carried out.

It is fully recognised that many of the experiments and accompanying notes could have been elaborated with advantage, but the necessity of restricting apparatus to the limits which it is possible to supply for the use of large classes, and the fact that the notes are only intended as laboratory guides, and not to supersede lectures, have determined the present scope of the volumes.

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1 *October* 1919.

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CONTENTS

I

COMPOSITION OF ORGANIC COMPOUNDS

1. In a small porcelain crucible ignite in turn small quantities of (a) sugar, (b) starch and (c) naphthalene. Note that in all three cases the substance chars at first and afterwards burns away completely, leaving no residue. (See note a.)

2. Repeat Expt. 1 using this time small quantities of (a) casein, (b) hay and (c) oil cake. Note that in these cases, while the greater portion of the material burns away, a small quantity of ash is left behind. (See note a.)

3. Well mix a small quantity of sugar with copper oxide and place the mixture in a dry test-tube. Fit the test-tube with a cork and a small leading tube bent once at right angles. Holding the test-tube obliquely, place a small quantity of anhydrous copper sulphate in the upper part of the tube near the cork and, having replaced the cork and leading tube, allow the latter to dip into lime-water contained in a second test-tube. With the tubes in the position indicated, strongly heat the mixture of sugar and copper oxide. Note that water is formed and collects in the cooler parts of the first test-tube, where it turns the white anhydrous copper sulphate blue, while, at the same time, the lime-water in the second test-tube turns milky. (See note b.)

4. Boil with dilute caustic soda solution in a test-tube small quantities of (a) oxamide and (b) casein. Test the steam evolved with red litmus paper and by the smell, and note that while ammonia is evolved from the oxamide, no ammonia is evolved from the casein. (See note c.)

5. Repeat Expt. 4 but instead of using caustic soda solution use dry powdered soda-lime intimately mixed with the organic substance. Note that in this case ammonia is evolved from both substances. (See note c.)

6. In a *small* test-tube heat up a small quantity of casein with two or three *very small* pieces of metallic sodium. When all reaction has finished, remove the test-tube from the flame, allow to cool slightly but, while still hot, drop the test-tube into cold water in a small porcelain dish. (*Stand well back from the dish while this portion of the experiment is being done.*) The test-tube on touching the cold water will crack and is then broken up into small pieces with the help of a rod, so that the water comes into contact with all the charred mixture in the tube. Filter the solution in the dish and divide into two portions. Saving one portion for Expt. 7, treat the other portion of the filtrate as follows. Add a few drops of ferrous sulphate solution

and a few drops of ferric chloride solution and boil. Cool and acidify with dilute hydrochloric acid. Note the greenish-blue or blue coloration or precipitate produced. (See note *d*.)

7. Using the second portion of the filtrate obtained in Expt. 6, divide it into three parts and carry out the following tests:

(*a*) Place a small quantity of the first part on a clean silver coin and note the black stain produced on standing.

(*b*) To the second part add acetic acid until acid and then lead acetate solution. Note the black precipitate produced.

(*c*) To the third part add a few drops of sodium nitroprusside solution and note the violet coloration produced. (See note *e*.)

8. In a porcelain crucible place a small quantity of a mixture of equal portions of potassium carbonate and potassium nitrate. Heat the mixture over the bunsen flame until fused and add to the fused mass, in small portions at a time, a small quantity of casein. When all the casein has been added, allow the mass to cool and dissolve out the mixture from the crucible by boiling crucible and contents in a beaker containing enough water to just cover the crucible. Filter the solution obtained and test the filtrate for sulphate and phosphate by the methods described in Volume I. Note the presence of both sulphate and phosphate in the liquid. (See note *f*.)

NOTES

Note a. Most pure organic bodies burn away completely on being ignited strongly. In the first stages of the ignition a black residue of carbon may be produced, but further ignition will remove this as the carbon is oxidized to carbon dioxide. Naturally occurring organic substances will, in almost all cases, leave a varying amount of ash and examples of this are the substances mentioned in Expt. 2. This ash is generally an impurity but in a few cases may be an essential constituent of the organic body. An extreme case of this is where a metallic salt of an organic acid is under examination. Here the amount of ash may be quite large and will generally consist of the oxide or carbonate of the metal concerned.

Note b. In this experiment the sugar on ignition reacts with the copper oxide. The hydrogen of the sugar forms water and the carbon, carbon dioxide, the necessary oxygen supplied by the copper oxide which breaks up leaving metallic copper. The production of water is demonstrated by the white anhydrous copper sulphate $CuSO_4$ taking up water and passing to the blue hydrated salt $CuSO_4\ 5H_2O$. The carbon dioxide produced reacts with the lime-water to form calcium carbonate as explained in Volume I.

Note c. While a few organic compounds containing nitrogen, of which oxamide is an example, liberate nitrogen as ammonia on boiling with aqueous soda, the greater number do not, and the presence of nitrogen can only be shown by strongly igniting the substance with soda-lime. Nearly all organic bodies of agricultural importance give off their nitrogen in the form of ammonia when heated with soda-lime. This test is not of universal application and the only satisfactory general test is the one described in Expt. 6.

Note d. This experiment depends on the production of sodium cyanide formed by the union of the sodium with the carbon and hydrogen in the organic substance. The cyanide so formed reacts with the ferrous hydroxide, formed when the ferrous sulphate is added to the alkaline solution, to give sodium ferro-cyanide which in turn reacts with the ferric chloride to give ferric-ferro-cyanide which is Prussian blue.

$$FeSO_4 + 2NaOH = Fe(OH)_2 + Na_2SO_4$$
$$Fe(OH)_2 + 6NaCN = Na_4Fe(CN)_6 + 2NaOH$$
$$3Na_4Fe(CN)_6 + 2Fe_2Cl_6 = Fe_4(Fe(CN)_6)_3 + 12NaCl$$
$$\text{(Prussian blue)}$$
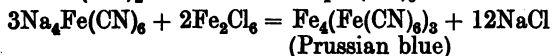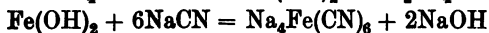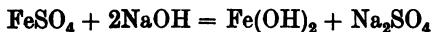
The depth of the colour produced will depend on the amount of nitrogen present. A very small quantity of nitrogen frequently gives only a greenish-blue coloration, a larger quantity a blue coloration, while a considerable quantity gives a blue precipitate.

Note e. On fusing with sodium any sulphur contained in an organic compound is converted into sodium sulphide. This will react with silver to form black silver sulphide, Ag_2S, with lead acetate to form black lead sulphide, PbS, and with sodium nitroprusside to give a purple colouring matter. The last-named test is particularly delicate.

Note f. An organic body containing sulphur or phosphorus reacts with the fused salts. The sulphur is oxidized to sulphate and the phosphorus to phosphate. These are then tested for in the ordinary way.

General note. The presence of halogens (= chlorine, bromine, iodine or fluorine) in organic compounds can be shown by heating the substance with metallic sodium as in the test for nitrogen and testing the solution for chlorides, bromides, etc. As however halogens containing organic substances are not often met with in agricultural chemistry, this experiment is not described in detail.

II

PURIFICATION OF ORGANIC COMPOUNDS

1. In the small distillation apparatus provided, place about 50 c.c. of the dirty mixture of alcohol and water. Heat gently and keep constant observation on the thermometer. Note that after a short period of heating the mixture begins to boil and that a considerable portion of the liquid will pass over while the thermometer stands at about 78° C. Collect this portion separately. After this portion has passed over, the thermometer will slowly rise and while the temperature is increasing in this way the liquid passing over should be collected separately in a second test-tube. When the temperature has reached about 100° C. the thermometer will again become almost constant and another large fraction, which should be collected in a third test-tube, will now pass over. The distillation may be carried on until only a very small quantity of liquid, together with the dirty impurities, remain in the flask. Test a small quantity of the liquid collected in the first test-tube as to whether it is inflammable or not and in this way show that it is alcohol. Test the liquid in the third test-tube and show that it will not burn and therefore it cannot be alcohol and is probably water only. (See note a.)

2. Purify the given sample of crude naphthalene in the following manner. Place some of the material in a small beaker and add a small quantity of alcohol. Warm up the mixture on a water bath and, when hot, filter rapidly through a dry filter paper and funnel into a second beaker. Allow to cool down and note that the naphthalene will now crystallise out. It should then be filtered off through another filter paper and finally allowed to dry by pressing it out on two or three filter papers and allowing it to stand until all the residual alcohol on the crystals has evaporated. If now the material is not, from its appearance, pure, the process should be repeated.

It should be noted that all heating of the alcoholic solution should be done on a water bath and not over a free flame. If the latter be used there is considerable danger of a fire taking place. (See note b.)

3. Check the purity of the given sample of acetone by introducing about 50 c.c. of this liquid into the distillation apparatus used in Expt. 1, warming up the flask and contents on a water bath and carefully reading the temperature at which the liquid distils over. Pure acetone boils at 56° C. and any liquid distilling more than 3° below or above this temperature should be rejected as an impurity. The pure acetone distilling about 56° C. should be carefully preserved and returned to the stock bottle. It must be remembered that acetone is inflammable and must only be distilled on a water bath while flames

must be kept away from the tube in which the distillate is being collected. (See note *c*.)

4. Check the purity of the given sample of naphthalene by finding its melting point. Take about 2 inches of the fine capillary tube provided and seal one end by fusing in the bunsen flame. Introduce a few finely divided particles of naphthalene into the open end of this tube and, by gently tapping, shake these down to the sealed end of the tube. The capillary tube is then moistened on the outside with water and the bulb end of a thermometer is also wetted. By simply placing the capillary tube in contact with the thermometer the two will be found to adhere quite easily and the capillary is arranged so that the sealed end containing the naphthalene is about half way down the bulb of the thermometer. The thermometer with its adherent capillary is then clamped or suspended so that its bulb is well covered with water contained in a small beaker, the latter being supported on gauze on a retort ring or tripod. The water is now heated by a *small* bunsen flame and is kept stirred carefully and constantly by a glass stirrer. The naphthalene in the capillary is watched constantly and directly it is seen to melt the temperature is read. Pure naphthalene melts at 80° C. (See note *d*.)

NOTES

Note a. This experiment is an illustration of what is termed fractional distillation. If a mixture of substances, whose boiling points are sufficiently far removed from one another, is treated in the way described they can generally be more or less completely separated from one another. In this case alcohol boils at 78° C., while water boils at 100° C. On distillation three fractions are obtained, a large one, containing almost wholly alcohol, boiling about 78° C., a small one, containing both alcohol and water, which passes over while the temperature is rising from about 78° to nearly 100° C., and a third large fraction, consisting almost entirely of water, which passes over at about 100° C.

If the two main fractions are again separately distilled, a further purification can be effected and pure alcohol and pure water obtained.

The non-volatile impurities, the "dirt," are left behind in the flask at the first distillation and give no further trouble.

Note b. This method of purification of a compound is a very general one. The first filtration gets rid of all insoluble impurities while the crystallisation itself tends to separate any different bodies which have dissolved in the solvent. On crystallisation taking place the least soluble of the bodies, which may be present in solution, will separate out in quantity while the more soluble bodies will remain behind in the solution. Only a partial separation of two substances may be effected by the first crystallisation but repetition of the process will, in the large majority of cases, eventually yield a pure product.

The solvent used must, of course, be chosen to suit the material to be purified and the following are very frequently employed, alcohol, acetone, benzene, chloroform, ethyl acetate, acetic acid, and, of course, water.

Note c. This method of checking the purity of a substance is very frequently used. The boiling points of all the common volatile organic compounds are known and the purity of a sample can be checked by comparing its boiling point with that found for carefully purified material. Again, the first step in the identification of an unknown liquid is frequently the finding of its boiling point and the comparison of this with the boiling point of known liquids. Boiling points frequently needed are ethyl ether 34° C., acetone 56° C., methyl alcohol 66° C., ethyl alcohol 78° C., benzene 80° C., acetic acid 118° C.

Note d. The determination of the melting point is a very general method of checking the purity of a solid substance, or of aiding the

identification of an unknown pure substance. The presence of impurities lowers the melting point, quite a small quantity of impurity frequently bringing down the melting point considerably. If the melting point of the material under investigation should be above 90° C., some other liquid than water must be used in the beaker, sulphuric acid or glycerine being the two most frequently employed.

III

HYDROCARBONS

1. Mix intimately one part of fused sodium acetate and three parts of soda lime. Place a quantity of this mixture in a boiling tube fitted with a cork and leading tube arranged as for the collection of gases over water. Heat the mixture strongly and collect a few boiling tubes full of the gas evolved. Note that the gas is colourless, without smell, and is not soluble in water. Holding one tube of the gas inverted apply the mouth of the tube to the bunsen flame and note that the gas burns with a flame resembling that of hydrogen. Replacing the leading tube previously used with a glass tube bent once at right angles, pass some of the gas, as it is being evolved, into dilute bromine water and note that no reaction takes place. (See note *a*.)

2. From the apparatus set up in the fume cupboard collect over water one jar of acetylene. Test it for inflammability by applying the bunsen flame to the mouth of the jar and note that it burns with a very luminous smoky flame.

From the same apparatus bubble a quantity of the gas through a small quantity of bromine water contained in a test-tube. Note that the bromine water is decolorised.

Repeat the last experiment but use a solution of cuprous chloride in ammonia instead of bromine water. Note the production of a brownish-red precipitate of copper acetylide. (*This precipitate must not be filtered off but must be decomposed by adding to the test-tube strong hydrochloric acid when it will be noticed that acetylene is liberated from it. In the dry condition copper acetylide is highly explosive.*) (See note *b*.)

3. Examine small quantities of refined petroleum and paraffin wax and, in separate test-tubes, heat small quantities of each with, (*a*) strong sulphuric acid, (*b*) strong nitric acid, (*c*) caustic soda solution. Note that in no case does any reaction occur. (See note *a*.)

4. Examine small quantities of the hydrocarbons, benzene, toluene and naphthalene. Note that all of them have characteristic odours, are insoluble in water and burn with smoky flames. (See note *c*.)

5. To a mixture of 2 c.c. of strong sulphuric acid and 1 c.c. of strong nitric acid, add a few drops of benzene and warm. Note that a reaction takes place. Dilute considerably with water and observe that a new product is evidently present in the liquid, as this now smells strongly of almonds. (See note *c*.)

NOTES

Note a. The gas evolved is methane or marsh gas, the sodium acetate, on heating, breaking up according to the equation:

$$CH_3.COONa \ + \quad NaOH \quad = \quad CH_4 \ + \quad Na_2CO_3$$
Sodium Acetate + Caustic Soda = Methane + Sodium Carbonate
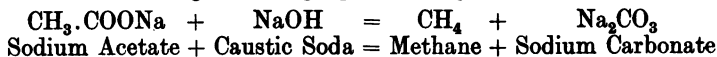
The caustic soda required is supplied by the soda-lime. Methane is the lowest member of a series of hydrocarbons with the general formula C_nH_{2n+2}. The lowest members, which are all gases, are:

<div style="text-align:center">

Methane, CH_4,

Ethane, C_2H_6,

Propane, C_3S_8,

Butane, C_4H_{10}.

</div>

The succeeding members of the series from C_5H_{12} to $C_{16}H_{34}$ are colourless liquids, while from $C_{17}H_{36}$ onwards the members of this series are colourless waxy solids.

Many members of the series occur in nature. Thus methane is formed by the decomposition of vegetable matter at the bottom of ponds (hence the name marsh gas) and is formed in the intestines of animals, especially in ruminants, by the decomposition of cellulose by bacteria. Petrol, paraffin oil and most lubricating oils are mixtures of varying composition of the liquid members of the series while vaseline and paraffin wax are mixtures of still higher members of the series.

All members of this series of hydrocarbons are very stable compounds being either not attacked or only attacked with difficulty by the most powerful chemical reagents. This stability is largely the result of these hydrocarbons containing enough hydrogen to satisfy all the valencies of the carbon, so that these compounds contain no double or treble bonds. Compounds like these containing only single bonds are known as *saturated* compounds.

Note b. The acetylene is produced by the action of water on calcium carbide, a reaction taking place which can be represented by the equation:

$$CaC_2 \qquad + \ 2H_2O \ = \quad C_2H_2 \quad + \qquad Ca(OH)_2$$
Calcium Carbide + Water = Acetylene + Calcium Hydroxide
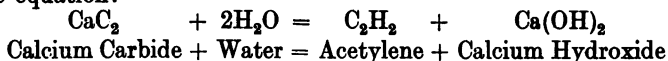
Acetylene is an example of an *unsaturated* hydrocarbon, that is of a hydrocarbon without sufficient hydrogen to satisfy all the carbon valencies and consequently containing bonds other than single ones. This particular compound contains a treble bond and is written constitutionally $CH{\equiv}CH$. Like all unsaturated compounds it is much more reactive than the saturated ones, especially towards the halogens, of which it readily adds on four atoms to form saturated com-

pounds. In the example given, four atoms of bromine are added on to give the compound acetylene tetrabromide:

$$C_2H_2 + 2Br_2 = C_2H_2Br_4$$

Acetylene + Bromine = Acetylene Tetrabromide
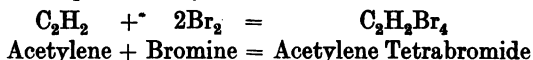
Another example of the reactivity of acetylene is given in the production of copper acetylide, C_2Cu_2, by passing the gas into ammoniacal cuprous chloride. Acetylene reacts with many metallic salts in this way giving products which are generally explosive.

Acetylene is the lowest member of a series of the general formula C_nH_{2n-2}, the other members of which are not technically important.

Between the saturated paraffins with the formula C_nH_{2n+2} and the very unsaturated acetylenes with the formula C_nH_{2n-2}, another important series of hydrocarbons are known with the general formula C_nH_{2n}. These are known as the olefines but, owing to the difficulty of their preparation, no example is given for laboratory work.

Note c. Benzene, toluene and naphthalene belong to a different type of hydrocarbon to those already mentioned. In the paraffins and acetylenes, for example, all the carbon atoms are in one long chain whereas in benzene, toluene, and naphthalene, the carbon atoms are in rings. The three hydrocarbons can be represented by the constitutional formulae:

Benzene   Toluene   Naphthalene
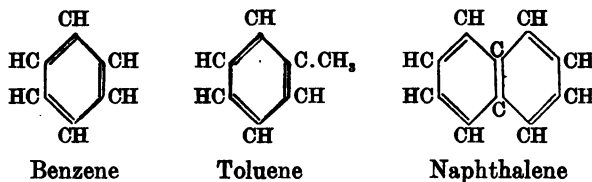
All chain compounds are known by the general term aliphatic compounds, whereas the ring compounds are termed aromatic compounds.

The reactivity of the aromatic compounds is fairly well marked especially in reactions where hydrogen is replaced by some other element, or group of elements, leaving the carbon ring intact. In the example given in Expt. 5 one hydrogen atom is replaced by the group $—NO_2$, with the production of nitrobenzene $C_6H_5.NO_2$, an oily liquid with the smell of almonds.

IV

ALCOHOLS (1)

1. **Methyl Alcohol, CH_3OH.** Examine a small quantity of this compound and carry out the following tests on it:

(a) Mix a small quantity with water and show that it is miscible with water in all proportions.

(b) Set fire to a few drops in a small porcelain dish and note that it burns with a pale non-luminous flame.

(c) Test it with litmus paper and note its neutral reaction.

(d) Into about 1 c.c. of methyl alcohol drop a very small piece of sodium. Note that a vigorous reaction takes place and that hydrogen is liberated.

(e) Warm about 1 c.c. of methyl alcohol with a small quantity of sodium salicylate and about 0·5 c.c. strong sulphuric acid. Note the characteristic smell of oil of wintergreen. (See general note and note a.) •

2. **Ethyl Alcohol, C_2H_5OH.** Examine a small quantity of this compound and carry out the following tests on it:

(a) Mix a small quantity with water and show that it is miscible with water in all proportions.

(b) Set fire to a few drops in a porcelain dish and note that it burns with a pale non-luminous flame.

(c) Test it with litmus paper and note that it has a neutral reaction.

(d) Warm about 1 c.c. with an equal quantity of glacial acetic acid and about 0·5 c.c. of strong sulphuric acid. Note the characteristic odour of ethyl acetate.

(e) To a few drops of alcohol add 5 c.c. of water and then iodine until a brown colour is produced. Now add sodium carbonate solution, drop by drop until the iodine colour just disappears. On gently warming a yellow precipitate, with the characteristic odour of iodoform, will be produced.

(f) To a little alcohol in a test-tube add a few drops of potassium bichromate solution and a small quantity of dilute sulphuric acid. Acetaldehyde, which can be recognised by its characteristic odour, will be produced and the yellow colour of the bichromate will be turned to green. (See general note and note b.)

3. Distil some beer in the apparatus provided and collect the first few c.c. of the distillate, noting that this passes over considerably

below 100° C. On this distillate carry out the iodoform test, as described in Expt. 2 (e) above and show by this means that the distillate contains alcohol.

4. In a small flask place some of the sugar solution provided and to this add a small quantity of yeast. Close the mouth of the flask with a plug of cotton wool and allow to stand in a warm place till next time. (See note c.)

5. Examine a small quantity of amyl alcohol and try on it the tests described in Expt. 2 (a), (b), (c) and (d) above. Note that this alcohol is not miscible with water and that the smell of amyl acetate produced in (d) is similar to, but can be distinguished from, that of ethyl acetate. (See note d.)

NOTES

General note. The compounds dealt with in this chapter belong to a series having the general formula $C_nH_{2n+1}OH$. This series contains the important members methyl alcohol, CH_3OH, ethyl alcohol, C_2H_5OH, propyl alcohol, C_3H_7OH, butyl alcohol, C_4H_9OH, and amyl alcohol, $C_5H_{11}OH$. Moreover, higher in the same series occur such solid alcohols as cetyl alcohol, $C_{16}H_{33}OH$, and myricyl alcohol, $C_{30}H_{61}.OH$. It will be noticed that all the compounds mentioned conform to the general formula given above and that they all contain a hydroxyl group (—OH), this being the characteristic group in all alcohols.

The alcohols are of common occurrence in nature and in the arts. Thus methyl alcohol occurs in oil of wintergreen (methyl salicylate) and is a bye-product in the distillation of wood. Ethyl alcohol occurs in all alcoholic liquors being produced there by the action of yeast on sugars. Amyl alcohol occurs in fusel oil, a bye-product in the manufacture of whiskey. The higher alcohols, like cetyl and myricyl alcohols, occur in the natural waxes, cetyl occurring in spermaceti and myricyl in beeswax.

Although they differ in appearance and physical properties, the alcohols resemble one another closely in chemical properties and these properties are conveniently described therefore in a general manner. Thus all the alcohols are inflammable, the carbon burning to carbon dioxide and the hydrogen giving water. They are all bodies neutral to litmus. The hydrogen atom in the hydroxyl group is replaceable, in all cases, by metals like sodium and potassium; thus with methyl alcohol, the following reaction takes place with sodium:

$$2CH_3OH \quad + \quad 2Na \quad = \quad 2CH_3.ONa \quad + \quad H_2$$
Methyl Alcohol + Sodium = Sodium Methylate + Hydrogen

while with ethyl alcohol an exactly similar reaction takes place, sodium ethylate being formed instead of sodium methylate.

All the alcohols react with organic acids to form compounds known as esters, these generally being oily compounds with characteristic odours. (They are dealt with fully in Chap. X.) Thus with salicylic acid, methyl alcohol gives methyl salicylate; ethyl alcohol and acetic acid gives ethyl acetate, while amyl alcohol and acetic acid gives amyl acetate. The particular reactions are dealt with in the special notes below.

Note a. In Expt. 1 (*d*) the reaction that takes place is represented by the equation:

$$CH_3OH \quad + C_6H_4.OH.COOH = C_6H_4.OH.COOCH_3 + \quad H_2O$$
Methyl Alcohol + Salicylic Acid = Methyl Salicylate + Water
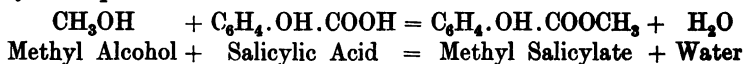
Note b. In Expt. 2 (*d*) the reaction is represented by the equation
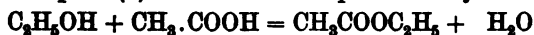
$$C_2H_5OH + CH_3.COOH = CH_3COOC_2H_5 + H_2O$$
Ethyl Alcohol + Acetic Acid = Ethyl Acetate + Water

Expt. 2 (*e*), by which iodoform is produced, can be represented by the scheme

$$C_2H_5OH   +   I_2   \longrightarrow   CH.I_3$$
Ethyl Alcohol + Iodine $\longrightarrow$ Iodoform

Expt. 2 (*f*) shows the oxidation of ethyl alcohol to acetaldehyde and can be represented by the equation

$$C_2H_5OH   +   O   =   CH_3.CHO + H_2O$$
Ethyl Alcohol + Oxygen = Acetaldehyde + Water

The oxygen is supplied by the potassium bichromate, which is itself reduced, giving, amongst other products, green chromium sulphate.

Note c. The reaction taking place in this fermentation is dealt with in the next chapter.

Note d. The reaction between the amyl alcohol and acetic acid is represented by the equation
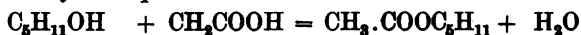
$$C_5H_{11}OH   + CH_3COOH = CH_3.COOC_5H_{11} + H_2O$$
Amyl Alcohol + Acetic Acid = Amyl Acetate + Water

V

ALCOHOLS (cont.). PHENOLS

1. Carefully take out the plug of cotton-wool from the flask in Expt. 4, Chap. IV, which was started last time, and, without pouring out any of the liquid, pour some of the gas contained therein into a boiling tube containing lime water. Shake up the gas with the lime water and notice that it goes milky, showing that the gas is carbon dioxide.

Distil the liquid in the flask as in Expt. 3 of the last chapter and test the first few c.c. of distillate for alcohol by the iodoform test. (See note a.)

2. Examine a small quantity of glycerol and try the following tests on it:

(a) To a few c.c. of water in a test-tube add small quantities of glycerol at a time and note that the two liquids are miscible in all proportions.

(b) To about 0·5 c.c. of glycerol in a test-tube add about twice its bulk of powdered potassium hydrogen sulphate. Heat the mixture in the bunsen flame and note the pungent smell of acrolein produced.

(c) To a few drops of glycerol in a test-tube add about 1 c.c. of strong sulphuric acid and gently heat the mixture. Note that acrolein is produced as in (b) and that in this case the glycerine is charred. (See note b.)

3. Examine a small quantity of phenol and try the following tests on it:

(a) To about 2 c.c. of water in a test-tube add very small quantities of crystalline phenol at a time. Note that, at first, the particles dissolve giving a solution of phenol, but that very soon the solution becomes saturated and, after this point is reached, the excess of phenol collects as an oily layer at the bottom of the water. When this oily layer has become noticeable, add an excess of dilute caustic soda solution and note that the oil dissolves. (*Phenol must not be allowed to come into contact with the skin or blistering will result. It must only be handled with a spatula.*)

(b) To a very dilute solution of phenol in water add a small quantity of ferric chloride solution and note the violet coloration produced. Show that this is destroyed by adding either acetic or hydrochloric acid.

(c) To a dilute solution of phenol add bromine water and note the yellow precipitate produced.

(d) Boil a few c.c. of a solution of phenol with Millon's reagent and note that a yellow precipitate is formed which dissolves in nitric acid giving a deep red colour.

(e) Heat a.fragment of phenol in a dry test-tube. Note that it volatilises and crystallises out in the cooler parts of the tube. (See note c.)

4. Examine the general appearance of the specimen of cholestrol and the shape of its crystals on the microscope slide arranged for you. (See note a.)

NOTES

Note a. Several different sugars will ferment with yeast, ethyl alcohol and carbon dioxide being produced as the main products in all cases. If dextrose or levulose be the sugar used the change can be represented by the equation:

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$
Dextrose or Levulose = Ethyl Alcohol + Carbon Dioxide

If, as in many cases, maltose is the sugar present, the change can be represented by the equation

$$C_{12}H_{22}O_{11} + H_2O = 4C_2H_5OH + 4CO_2$$
Maltose + Water = Ethyl Alcohol + Carbon Dioxide
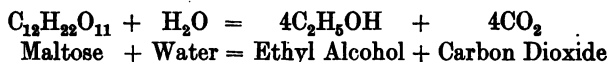
Note b. Glycerol or glycerine differs from the alcohols dealt with up to now in that it contains three hydroxyl groups instead of only one. It can be represented by the formula $C_3H_5(OH)_3$. It occurs in all animal and vegetable fats, a point which will be dealt with later in dealing with these bodies. In Expt. 2 (*b*) and (*c*) a most characteristic reaction of glycerine is shown. The potassium hydrogen sulphate, or the sulphuric acid, reacts with the glycerine, abstracting two molecules of water from one molecule of glycerine and forming the pungent smelling compound acrolein, $C_2H_3.CHO$.

$$C_3H_5(OH)_3 = C_2H_3.CHO + 2H_2O$$
Glycerol = Acrolein + Water
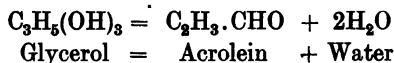
This reaction takes place to some extent when fats are burnt and helps to give rise to the disagreeable odour.

Note c. Phenol, also known as carbolic acid, belongs to another class of hydroxy compounds which are not alcohols. The term phenol is used to describe the hydroxy compounds of benzene and its related compounds. The relation of the commonest of these bodies, generally called phenol, to benzene is shown by the following formulae:
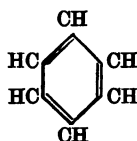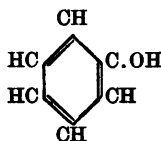
Benzene   Phenol

As shown in Expt. 3 (*a*) it is moderately soluble in water and easily soluble in caustic soda solution, the increased solubility in the latter

case being due to the formation of the sodium salt of phenol, $C_6H_5 \cdot ONa$. It is readily volatile, Expt. 3 (e), and reacts with bromine, Expt. 3 (c), to give a yellow precipitate of tri-bromphenol, $C_6H_2Br_3OH$.

Note d. Cholestrol, $C_{27}H_{45}OH$, is a mono-hydroxy alcohol of unknown constitution. This compound, or very similar bodies, occur in small quantities in almost all animal and vegetable oils and fats and in animal products such as gall stones, brain matter, etc.

VI

ALDEHYDES

1. (a) Heat a few drops of methyl alcohol with potassium bichromate solution and a small quantity of strong sulphuric acid. Note the pungent characteristic odour of formaldehyde.

(b) Place a small wad of asbestos at the bottom of a test-tube and on this drop two or three drops of methyl alcohol. Strongly heat a piece of copper oxide in the outer part of the bunsen flame and then quickly drop this on to the asbestos in the test-tube. Notice that the surface of the copper oxide is reduced to metallic copper and that the smell of formaldehyde can be noticed in the tube. (See general note and note a.)

2. Examine a few c.c. of commercial formalin solution, note its characteristic smell and carry out the following tests with it:

(a) Evaporate a small quantity to dryness in a porcelain dish on a water bath in a fume cupboard. Note the white solid residue remaining.

(b) To a small quantity of silver nitrate solution add ammonia drop by drop until the precipitate which first forms just re-dissolves. To this reagent add a small quantity of a solution made by very largely diluting a few drops of formalin with distilled water. Warm the mixture by standing the test-tube in a beaker of water and gently heating it with the bunsen flame. Note that metallic silver is produced either as a grey powder or, if the solutions be sufficiently dilute and the test-tube scrupulously clean, as a silver mirror adherent to the side of the tube.

(c) Just decolorise a small quantity of magenta solution by bubbling sulphur dioxide gas from a siphon into it. To this reagent add a few drops of a solution of formalin in water and note that the red colour is restored.

(d) To a small quantity of formalin solution in a test-tube add caustic soda solution and boil. Note the formation of an oily substance which becomes resinous on cooling.

(e) To a few c.c. of Fehling's solution add a small quantity of formalin solution and warm. Note the formation of a red precipitate. (See general note and note b.)

3. To a little potassium bichromate solution in a test-tube add a little strong sulphuric acid and shake. To the mixture, while still hot, add a few drops of ethyl alcohol and note that the yellow colour of the bichromate is changed to green, while the characteristic fruity

3—2

smell of acetaldehyde can be noticed in the test-tube. (See general note and note c.)

4. Try the tests described in Expt. 2 (b), (c), (d), and (e) using a dilute solution of acetaldehyde instead of formalin solution. (See note d.) Also try the following experiments on acetaldehyde:

(a) Shake a few c.c. of acetaldehyde with an equal quantity of a saturated solution of sodium bisulphite, and note that a crystalline precipitate is produced.

(b) To about five drops of phenylhydrazine add 3 or 4 c.c. of water and then glacial acetic acid, drop by drop, until the oily drops of phenylhydrazine dissolve. To this reagent add about 2 c.c. of a dilute solution of acetaldehyde, shake well and gently warm the mixture. Notice the separation of an oil which may solidify on cooling. (See note e.)

5. Try Expt. 4 (a) and (b) and Expt. 2 (b) using benzaldehyde instead of acetaldehyde or formaldehyde. Note that in this case the product formed with phenylhydrazine separates as a crystalline solid. (See note f.)

6. Try Expt. 2 (b) and (e) and Expt. 4 (b) using an aqueous solution of dextrose instead of the aldehydes already mentioned. (See note g.)

NOTES

General note. The aldehydes are that class of organic compounds which are characterised by containing the group —CHO. The aldehydes dealt with in this chapter are formaldehyde $H.CHO$, acetaldehyde $CH_3.CHO$, benzaldehyde $C_6H_5.CHO$, and dextrose $CH_2.OH.(CHOH)_4.CHO$. The aldehydes are formed by the oxidation of the alcohols, a —CH_2OH group in the latter losing two atoms of hydrogen and becoming the —CHO group, while they are generally easily oxidised to organic acids, the —CHO group adding on one atom of oxygen and becoming a —COOH group, the characteristic group of the acids.

It is the —CHO group which gives the aldehydes most of their characteristic properties, the ease with which this group is oxidised making them powerful reducing agents. This reducing property of the aldehydes will be shown in several of the experiments described.

Note a. In Expt. 1 (*a*) the methyl alcohol is oxidised to formaldehyde by the bichromate, which is a powerful oxidising agent, the bichromate being itself reduced giving, amongst other substances, green chromium sulphate.

$$CH_3OH \quad + \quad O_2 \quad = \quad H.CHO \quad + \quad H_2O$$
Methyl Alcohol + Oxygen = Formaldehyde + Water
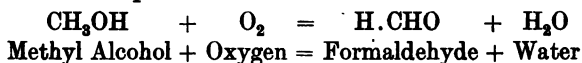
In Expt. 1 (*b*) the same reaction takes place, the oxygen in this case being supplied by the copper oxide, CuO, which is itself reduced to metallic copper, Cu.

Note b. In Expt. 2 (*a*) the formaldehyde, $H.CHO$, is polymerised on evaporation to solid paraformaldehyde, probably $(H.CHO)_3$.

Expt. 2 (*b*) shows the reduction of silver oxide, Ag_2O, to metallic silver, the formaldehyde being itself oxidised to formic acid.

$$Ag_2O \quad + \quad H.CHO \quad = \quad H.COOH \quad + \quad 2Ag$$
Silver Oxide + Formaldehyde = Formic Acid + Silver

Expt. 2 (*e*) shows the reduction of cupric oxide, CuO, to red cuprous oxide, Cu_2O, the aldehyde being oxidised to formic acid, $H.COOH$ (Fehling's solution can be considered as an alkaline solution of cupric oxide, CuO).

$$2CuO \quad + \quad H.CHO \quad = \quad H.COOH \quad + \quad Cu_2O$$
Cupric Oxide + Formaldehyde = Formic Acid + Cuprous Oxide
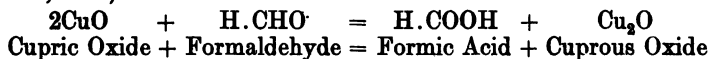
Note c. In this experiment ethyl alcohol is oxidised to acetaldehyde by the bichromate in a similar manner to that already described for methyl alcohol.

$$C_2H_5OH \quad + \quad O_2 \quad = \quad CH_3.CHO \quad + \quad H_2O$$
Ethyl Alcohol + Oxygen = Methyl Alcohol + Water
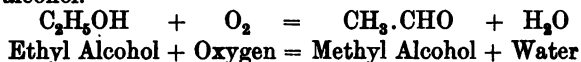
Note d. The explanation of these tests is similar to that given for formaldehyde. Thus with the ammoniacal silver nitrate solution, silver oxide is reduced by the acetaldehyde to metallic silver according to the following equation, the aldehyde being oxidised to acetic acid:

$$Ag_2O \ + \ CH_3.CHO \ = CH_3.COOH + 2Ag$$
Silver Oxide + Acetaldehyde = Acetic Acid + Silver
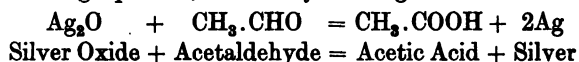
With Fehling's solution, the aldehyde is also oxidised to acetic acid, the cupric oxide of the Fehling's solution being reduced to red cuprous oxide.

$$2CuO \ + \ CH_3.CHO \ = CH_3.COOH + \ Cu_2O$$
Cupric Oxide + Acetaldehyde = Acetic Acid + Cuprous Oxide
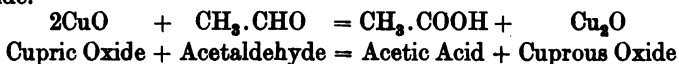
Note e. Many aldehydes form crystalline additive compounds with sodium bisulphite. In Expt. 4 (*a*) the following reaction takes place:

$$CH_3.CHO + NaH.SO_3 = CH_3.CH.OH.SO_3Na$$
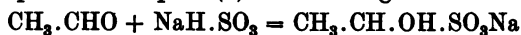
Expt. 4 (*b*) illustrates a general reaction for aldehydes. The aldehyde reacts with the phenylhydrazine with the elimination of water and the formation of a body known as a hydrazone.

$$CH_3.CHO \ + \ C_6H_5.NH.NH_2 \ = \ CH_3.CH:N.NH.C_6H_5 \ + \ H_2O$$
Acetaldehyde + Phenylhydrazine = Acetaldehyde Hydrazone + Water
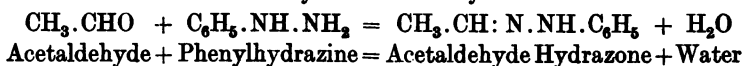
Note f. The reactions of benzaldehyde in Expt. 5 are exactly similar to those already described for other aldehydes. They can be represented by the following equations:

$$C_6H_5.CHO + NaH.SO_3 = C_6H_5.CH.OH.SO_3Na$$
$$C_6H_5.CHO + C_6H_5.NH.NH_2 = C_6H_5.CH:N.NH.C_6H_5 + H_2O$$
$$C_6H_5.CHO + Ag_2O = C_6H_5.COOH + 2Ag$$
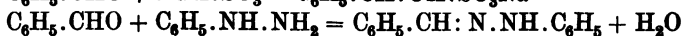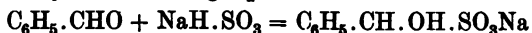
Note g. The explanation of the reactions in Expt. 6 is similar to that already given in the notes above, the aldehyde group in dextrose behaving similarly to that in, say, acetaldehyde or benzaldehyde.

VII

KETONES

1. Examine a few c.c. of acetone and note the characteristic odour. Try the following tests on it:

(a) Show that it is miscible with water in all proportions and, by burning a little in a porcelain dish, that it is inflammable.

(b) Show, by using a solution of acetone in water, that it does not reduce Fehling's solution, does not reduce ammoniacal silver nitrate solution, does not restore the colour to magenta decolorised by sulphur dioxide and does not give a resin with caustic soda. The requisite solutions for these tests are made as directed in the preceding chapter on aldehydes.

(c) To one or two c.c. of acetone add about an equal volume of saturated sodium bisulphite solution. Note that the mixture becomes warm and that on cooling the additive compound of acetone and sodium bisulphite crystallises out.

(d) To a few drops of phenylhydrazine in a test-tube add a few c.c. of water and then glacial acetic acid, drop by drop, until the oily drops just dissolve. To this reagent add a small quantity of a dilute solution of acetone and warm. Cool and note that an oily phenylhydrazone separates out. (See note a.)

2. In a boiling tube, fitted with a cork and a leading tube bent once at right angles, place about five grams of anhydrous calcium acetate. Heat with the bunsen flame and collect the distillate which passes over in a test-tube. Note that this distillate has the characteristic odour of acetone and, when diluted with water, will answer to tests given in Expt. 1 (c) and (d) above. (See note a.)

ETHERS

3. Examine a few c.c. of ether and note its characteristic odour. Try the following tests on it:

(a) Show that it is not miscible with water and that it is lighter than water, since it separates out on the surface as a separate layer.

(b) Show that it is very inflammable by burning a few drops in a porcelain dish.

(c) Show that it is a good solvent for oils in the following way. Shake up about 0·5 c.c. of linseed oil with water and note that the oil floats about as separate drops. Now add a few c.c. of ether and, closing the test-tube with the finger, shake up vigorously. Allow to

stand, and note that the ether has extracted all the oily globules from the water. Pour off the ether carefully into a porcelain dish and place this on a water bath. The ether will evaporate off leaving the oil. (See note *b*.)

4. Heat about six drops of absolute alcohol with three drops of strong sulphuric acid, in a test-tube. On cooling the odour of ether can be detected. (See note *b*.)

NOTES

Note a. The ketones are a group of organic bodies characterised by the general formula R.CO.R', where R and R' are hydrocarbon radicles and where these radicles may be the same or different. The commonest member of the group is acetone, $CH_3.CO.CH_3$, which occurs as a product of the distillation of wood and can also be prepared by heating calcium acetate when the following reaction takes place:

$$(CH_3.COO)_2Ca = CH_3.CO.CH_3 + CaCO_3$$

Calcium Acetate = Acetone + Calcium Carbonate

The ketones, while not showing the powerful reducing actions of the aldehydes (Expt. 1 (*b*)), resemble these bodies in many respects. Thus with bisulphite crystalline additional compounds are formed (Expt. 1 (*c*)).

$$CH_3.CO.CH_3 + NaHSO_3 = (CH_3)_2.C.OH.SO_3Na$$

Acetone + Sodium Bisulphite = Acetone Bisulphite,

while with phenylhydrazine, hydrazones are formed:

$$CH_3.CO.CH_3 + C_6H_5.NH.NH_2 = (CH_3)_2C = N.NH.C_6H_5$$

Acetone + Phenylhydrazine = Acetone Hydrazone
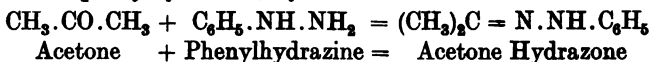
Another important body containing the ketone group is the sugar levulose, $CH_2OH.(CH.OH)_3.CO.CH_2OH$.

Note b. The ethers are a group of compounds characterised by the general formula, R.O.R', where R and R' are hydrocarbon radicles and may be identical or different. The commonest member is ethyl ether, $C_2H_5.O.C_2H_5$, the ordinary ether of the laboratory, which is prepared by heating alcohol with sulphuric acid (Expt. 4). The sulphuric acid can be considered to act as a dehydrating agent, removing one molecule of water from two molecules of alcohol.

$$2C_2H_5OH = C_2H_5.O.C_2H_5 + H_2O$$

Ethyl Alcohol = Ethyl Ether + Water
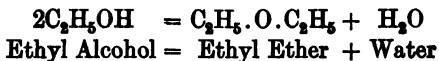
Ethyl ether is a colourless mobile liquid, not miscible with water, very inflammable, and whose chief value in the laboratory is that it is an excellent solvent for many organic substances, e.g. oils and fats, as illustrated by Expt. 3 (*c*).

VIII

ACIDS

1. **Formic Acid, H.COOH.** Examine the specimen of formic acid and note its pungent odour. Carry out the following tests on either a small quantity of formic acid or, more conveniently, on one of its salts, a formate:

(a) Heat a small quantity of formate with strong sulphuric acid in a test-tube. Apply a flame to the mouth of the test-tube and note that a gas is being evolved which burns with a blue flame.

(b) To a solution of a formate in water add silver nitrate solution. A white precipitate may be produced but, in any case, grey metallic silver will be precipitated on warming.

(c) To a solution of a formate in water add dilute sulphuric acid and boil. Note that the formic acid is volatile in steam as shown by the pungent odour of the latter. (See note a.)

2. **Acetic Acid, $CH_3.COOH$.** Examine a specimen of glacial acetic acid, note its characteristic odour and try the following tests on it:

(a) Mix a little with water and note that the two liquids are miscible in all proportions, and that when the solution is boiled the acetic acid can be smelt in the steam.

(b) Stand a test-tube, containing a little glacial acetic acid, in crushed ice and note that the acid solidifies, re-melting on removal from the ice, at about 17° C.

(c) Warm a drop of glacial acetic acid, or a small quantity of an acetate, with a little alcohol and some strong sulphuric acid. Note the pleasant and characteristic odour of ethyl acetate.

(d) To a solution of sodium acetate add a few drops of ferric chloride solution. Note that a red colour is produced and that, on boiling, a precipitate is thrown down. (See note b.)

3. **Butyric Acid, $C_3H_7.COOH$.** Examine the specimen of butyric acid noting its disagreeable odour and try the following test on it:

(a) To one drop of butyric acid add a few c.c. of water. Note that the acid is soluble and that, on boiling, the smell of butyric acid can be detected in the steam.

4. **Palmitic Acid, $C_{15}H_{31}.COOH$.** Examine the specimen of palmitic acid and note its general appearance. Carry out the following tests on it:

(a) To a small quantity in a test-tube add a few c.c. of water. Note that it is insoluble even on boiling. To the mixture of water and

acid, add caustic soda solution, warm and note that the acid passes into solution. Cool and acidify this solution with a dilute mineral acid and note that the palmitic acid is again precipitated.

(b) To a little palmitic acid in a test-tube add a few c.c. of ether and note that the acid is easily soluble in this solvent.

(c) Dissolve a little palmitic acid in chloroform and to this solution add a few drops of a chloroform solution of bromine. Note that no decolorisation of the bromine solution takes place and compare this with the same test carried out as described below on oleic acid. (See note c.)

5. Stearic Acid, $C_{17}H_{35}$.COOH. Examine the specimen of stearic acid and carry out on it all the tests described above for palmitic acid. (See note d.)

6. Oleic Acid, $C_{17}H_{33}$.COOH. Examine the specimen of oleic acid, noting that unlike palmitic and stearic acid it is a liquid, and carry out all the tests described in Expt. 4 for palmitic acid. Note particularly that, in this case, in the test with bromine in chloroform, the bromine is decolorised. (See note e.)

· NOTES

General note. The organic acids described in this chapter belong, with the exception of oleic acid, to a series of acids with the general formula, $C_nH_{2n+1}COOH$. They are a most important series of bodies, the best known members being as follows:

Formic Acid	...	$H.COOH$,
Acetic	,, ...	$CH_3.COOH$,
Propionic	,, ...	$C_2H_5.COOH$,
Butyric	,, ...	$C_3H_7.COOH$,
Valeric	,, ...	$C_4H_9.COOH$,

and the higher members of the series:

Palmitic Acid	...	$C_{15}H_{31}.COOH$,
Stearic	,, ...	$C_{17}H_{35}.COOH$.

All members contain the characteristic group —COOH, the carboxyl group, and all of them are saturated compounds. Oleic acid, $C_{17}H_{33}.COOH$, does not belong to this series but to one having the general formula $C_nH_{2n-1}.COOH$, and is an unsaturated compound. .

Note a. Expt. 1˙(a). The formic acid is broken up by strong sulphuric acid with the production of carbon monoxide, an inflammable gas:

$$2H.COONa + H_2SO_4 = 2CO + Na_2SO_4 + 2H_2O$$

Sodium	+ Sulphuric	= Carbon	+ Sodium	+ Water
Formate	Acid	Monoxide	Sulphate	
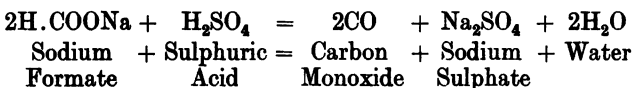
Expt. 1 (b). In this experiment silver formate is formed on adding silver nitrate to the formate solution but this is decomposed on warming with the production of metallic silver:

$$H.COOH + AgNO_3 = H.COOAg + HNO_3$$
$$2H.COOAg = H.COOH + CO_2 + 2Ag$$
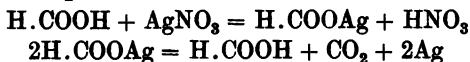
Note b. Expt. 2 (c). In this experiment the acetic acid reacts with alcohol to form ethyl acetate, water being eliminated.

$$CH_3.COOH + C_2H_5OH = CH_3.COOC_2H_5 + H_2O$$
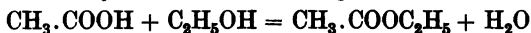
Expt. 2 (d). In this experiment the red coloration is due to the formation of red ferric acetate:

$$3CH_3.COONa + FeCl_3 = (CH_3.COO)_3Fe + 3NaCl$$
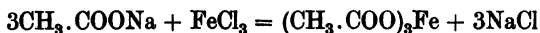
On boiling this ferric acetate is decomposed with the production of a basic ferric acetate, $(CH_3.COO)_3Fe.Fe_2O_3$, which is insoluble in water and is thrown down as a precipitate.

Note c. Expt. 4 (*a*). The palmitic acid is insoluble in water but soluble in soda solution to form sodium palmitate. This is decomposed by mineral acids with the reprecipitation of palmitic acid.

$$C_{15}H_{31}.COOH + NaOH = C_{15}H_{31}COONa + H_2O$$
$$C_{15}H_{31}.COONa + HCl = C_{15}H_{31}.COOH + NaCl$$
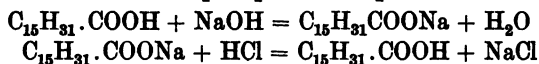
Expt. 4 (*c*). Palmitic acid being a saturated compound does not add on bromine and the solution therefore is not decolorised. Compare the similar experiment described under methane.

Note d. The explanation for these experiments is exactly similar to that given for palmitic acid in note *c* above.

Note e. The explanation of the experiments on oleic acid is similar to that given in note *c* above for palmitic acid, except that in this case, the oleic acid, being an unsaturated compound, adds on two atoms of bromine, and the bromine solution becomes decolorised. There is formed di-bromstearic acid and the reaction can be represented by the equation

$$C_{17}H_{33}.COOH + Br_2 = C_{17}H_{33}Br_2.COOH$$
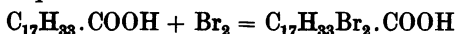
Compare a similar experiment described under acetylene

IX

ACIDS (cont.)

1. Benzoic Acid, $C_6H_5.COOH$. Examine a sample of benzoic acid, note its crystalline nature and try the following tests on a small quantity of it.

(a) To a few c.c. of water in a test-tube add a few particles of benzoic acid. Note that it is insoluble in cold water, that on heating it dissolves and that on cooling the solution the acid crystallises out once more.

(b) Warm small quantities of benzoic acid with caustic soda and sodium carbonate solutions. Note that the acid dissolves and does not crystallise out on cooling but that the addition of a dilute mineral acid precipitates benzoic acid once more. Shake up the solutions containing the suspended benzoic acid with ether and note that the acid dissolves in the ether and that if the latter be poured off and evaporated in a dish on a water bath, the benzoic acid is left as a crystalline residue.

(c) Heat a small quantity of benzoic acid in a dry test-tube and note that it sublimes, crystallising out again on the cooler parts of the tube.

(d) To a small quantity of benzoic acid add a little alcohol and a few drops of strong sulphuric acid. Warm and note the pleasant smell of ethyl benzoate. (See note a.)

2. Salicylic Acid, $C_6H_4.OH.COOH$. Examine a sample of salicylic acid, note its crystalline nature and try the tests given in Expt. 1 (a) and (b) on it. Also try the following tests:

(a) Heat a small quantity well mixed with soda lime and note that the smell of phenol is given off.

(b) To a salt of salicylic acid, say sodium salicylate, in aqueous solution add a few drops of ferric chloride solution and note the violet colour produced.

(c) To a small quantity of salicylic acid add methyl alcohol and a few drops of strong sulphuric acid. Warm and note the characteristic smell of methyl salicylate (oil of wintergreen). (See note b.)

3. Lactic Acid, $CH_3.CH.OH.COOH$. Examine the specimen of lactic acid and note that it is a syrupy liquid. Try the following tests on it:

(a) Taking a few drops at a time in a test-tube show that it is soluble in water, alcohol and ether.

(b) Boil 10–20 c.c. of lactic acid solution for some time with zinc carbonate. Filter while hot and evaporate to small bulk when the zinc salt of lactic acid will crystallise out. (See note c.)

4. Oxalic Acid, $(COOH)_2$. Examine a specimen of oxalic acid and try the following tests on it:

(a) Shake up a small quantity with water and note its ready solubility.

(b) Heat a small quantity of an oxalate or oxalic acid with strong sulphuric acid and show that both carbon monoxide and dioxide are given off by showing that the gases evolved are both inflammable and turn lime water milky.

(c) To a solution of an oxalate, say ammonium oxalate, add calcium chloride solution and note the white precipitate of calcium oxalate.

(d) Gently warm a small quantity of oxalic acid or an oxalate with dilute sulphuric acid and then add to the solution, drop by drop, a dilute solution of potassium permanganate. Note that the permanganate is decolorised. (See note d.)

5. Tartaric Acid, $(CH.OH.COOH)_2$. Examine a specimen of tartaric acid and try the following tests on it:

(a) Shake up with water and note that it is readily soluble.

(b) Heat a crystal of tartaric acid in a dry test-tube and note the charring and odour of burnt sugar.

(c) Heat a crystal of the acid with strong sulphuric acid. Note that the mixture blackens and that a considerable amount of gas (carbon monoxide and dioxide and sulphur dioxide) is given off.

(d) Heat a small fragment of tartaric acid with strong sulphuric acid containing 1 per cent. resorcinol, in a porcelain dish. Note the reddish violet colour produced.

6. Citric Acid, $CH_2.COOH.C(OH).COOH.CH_2COOH$. Examine the specimen of citric acid and try the tests given in Expt. 5 (a), (b) and (c) on it.
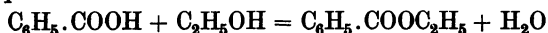
NOTES

General note. The acids described in this chapter belong to different groups. Benzoic acid, $C_6H_5.COOH$, is the simplest aromatic organic acid and can be considered as the body resulting from the replacement of one atom of hydrogen in the benzene ring by a carboxyl group, —COOH. Salicylic acid, $C_6H_4.OH.COOH$, is hydroxy-benzoic acid and is formed from benzene by the replacement of two hydrogen atoms from this compound, one by a hydroxy (—OH) group and one by a carboxyl (—COOH) group. It is therefore both a phenol and an acid at the same time. Lactic acid, $CH_3.CH.OH.COOH$, is hydroxy propionic acid, that is propionic acid, $CH_3.CH_2COOH$, in which one hydrogen atom is replaced by a hydroxyl (—OH) group. Oxalic acid, $(COOH)_2$, tartaric acid, $(CH.OH.COOH)_2$, and citric acid, $CH_2.COOH.C(OH).COOH.CH_2COOH$, are all examples of polybasic organic acids, that is they contain more than one hydrogen atom replaceable by metals to form salts. Oxalic acid for instance can give two potassium salts, acid potassium oxalate, $COOK.COOH$, and neutral potassium oxalate, $(COOK)_2$. Tartaric acid and citric acids are in addition hydroxy acids, that is, they contain —OH groups as well as carboxyl (—COOH) groups.

Note a. Expt. 1 (*b*). The benzoic acid dissolves in the alkali to give sodium benzoate, soluble in water. This is decomposed by the mineral acid and free benzoic acid is precipitated.

$$C_6H_5.COOH + NaOH = C_6H_5.COONa + H_2O$$
$$C_6H_5.COONa + HCl = C_6H_5.COOH + NaCl$$
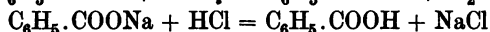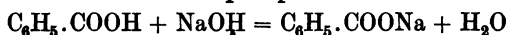
Expt. 1 (*d*). The ethyl benzoate is formed according to the following equation:

$$C_6H_5.COOH + C_2H_5OH = C_6H_5.COOC_2H_5 + H_2O$$

Note b. Expt. 2 (*a*). The salicylic acid is decomposed when heated with soda lime according to the equation

$$C_6H_4.OH.COOH + NaOH = C_6H_5.OH + Na_2CO_3$$
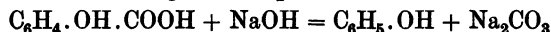
Expt. 2 (*c*). The methyl salicylate is produced according to the following equation:

$$C_6H_4.OH.COOH + CH_3OH = C_6H_4.OH.COOCH_3 + H_2O$$
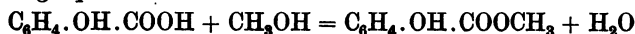
Note c. Expt. 3 (*b*). The following equation represents the formation of zinc lactate, the most characteristic salt of lactic acid.

$$2CH_3.CH.OH.COOH + ZnCO_3$$
$$= (CH_3.CH.OH.COO)_2Zn + CO_2 + H_2O$$
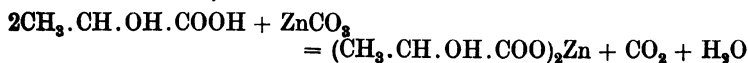
Note d. Expt. 4 (*b*). With sulphuric acid, oxalic acid decomposes in the following manner:

$$(COOH)_2 = CO_2 + CO + H_2O$$

Expt. 4 (*c*). The precipitation of calcium oxalate can be represented by the following equation:

$$(COONH_4)_2 + CaCl_2 = (COO)_2Ca + 2NH_4Cl$$
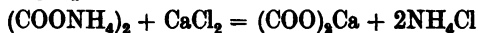
Expt. 4 (*d*). Oxalic acid is decomposed by permanganate in the presence of sulphuric acid according to the following equation:

$$5(COOH)_2 + K_2Mn_2O_8 + 3H_2SO_4 = K_2SO_4 + MnSO_4 + 10CO_2 + 8H_2O$$
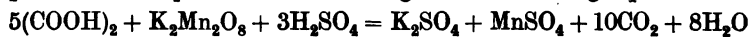
X

ESTERS

1. **Ethyl Acetate, $CH_3.COOC_2H_5$.** Prepare ethyl acetate in the following manner. A distilling flask of about 500 c.c. capacity is fitted to a condenser and a receiver, while through the cork in the neck of the flask passes a thermometer and dropping funnel. The flask stands in an oil bath which is itself supported on a tripod. In the flask place a mixture of 50 c.c. absolute alcohol and 50 c.c. strong sulphuric acid. The oil bath is now heated to 140° C. and is kept as near as possible to this temperature. A mixture of 100 c.c. of glacial acetic acid and 100 c.c. absolute alcohol is now run into the flask from the dropping funnel, drop by drop, and at the same rate as the liquid distils over and collects in the receiver. When all the liquid has been added in this way, the distillate is transferred to a flask and a strong solution of sodium carbonate added, small quantities at a time, with constant shaking, until the upper layer ceases to redden blue litmus. The liquid is then transferred to a separating funnel and the lower aqueous layer run off. The upper layer is transferred to a small flask, a few pieces of anhydrous calcium chloride added and the liquid allowed to stand for some time. The liquid is now poured off from the calcium chloride into a dry flask and is distilled from a water bath, the fraction distilling between 74–79° C., ethyl acetate, being collected separately and preserved. (See note *a*.)

2. Take 15–20 c.c. of the ethyl acetate prepared in Expt. 1, add three times its volume of caustic soda solution (1 part NaOH: 3 parts H_2O). Place the mixture in a small flask fitted to an upright condenser and heat over a wire gauze until the upper layer of ethyl acetate has disappeared. Disconnect the flask from the upright condenser and attach it to the condenser arranged obliquely, as for the ordinary distillation of liquids. Distil the liquid until about 20–30 c.c. have passed over and preserve this distillate. To the liquid left in the flask add a few drops of an indicator and then dilute sulphuric acid until just neutral.

Test the distillate for ethyl alcohol by the iodoform test given in Chap. IV and the neutralised liquid in the flask for acetate by the ferric chloride test given in Chap. VIII. (See note *b*.)

NOTES

General note. The esters are a group of bodies formed by the inter-action of an acid and alcohol with the elimination of water. Thus if an organic acid be represented by the general formula $R.COOH$ and an alcohol by the formula $R'.OH$, where R and R' are hydrocarbon radicles, the formation of an ester can be represented by the equation

$$R.COOH + R'.OH = R.COOR' + H_2O$$
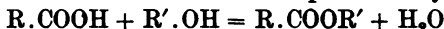
In this case the equation represents a monobasic acid, that is, an acid containing only one —COOH group, but a similar reaction will take place for each —COOH group, if the acid should contain more than one of these. Similarly if the alcohol concerned should contain more than one —OH group, it will be able to combine with more than one molecule of an acid containing only one —COOH group.

The esters are neutral bodies, many of them having pleasant odours, are generally insoluble in water and are capable of being broken up by various reagents into the acid and alcohol from which they were produced, the reverse reaction to the one shown in the equation above taking place. The reagents most frequently used are the caustic alkalis, and, taking the use of caustic soda as an example, a reaction represented by the following equation takes place:

$$R.COOR' + NaOH = R.COONa + R'.OH$$

That is, in this case, the sodium salt of the acid and the free alcohol are produced. This process is generally spoken of as *hydrolysis* or *saponification*.

Note a. For elementary purposes the reaction between the reagents in this experiment can be considered to be represented by the equation

$$CH_3COOH + C_2H_5OH = CH_3COOC_2H_5 + H_2O$$
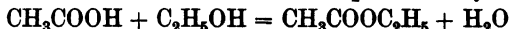
the sulphuric acid serving to cause the elimination of the molecule of water. The distillate first obtained contains small quantities of acetic acid, sulphur dioxide, etc., in addition to the ethyl acetate and these impurities are removed by the washing with sodium carbonate. After separating the ester layer it still contains small quantities of water and this is removed by the anhydrous calcium chloride which combines with the water to give the crystalline salt, $CaCl_2.6H_2O$. The final distillation gives the pure dry ester.

Note b. Expt. 2 illustrates the hydrolysis of the ester made in Expt. 1. The ethyl acetate is broken up by the caustic soda according to the following equation:

$$CH_3.COOC_2H_5 + NaOH = CH_3.COONa + C_2H_5OH$$
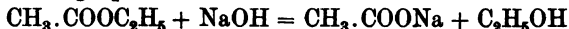
The distillation drives over the alcohol while the sodium acetate remains in the flask with an excess of caustic soda. By neutralising the excess of soda, the acetate can be tested for without it being further separated.

XI

ESTERS (FATS)

1. Place about 10–15 grs. of tallow in a small flask, add about 50 c.c. of a 10% solution caustic soda and boil until the whole of the fat has disappeared. Cool, add a few drops of phenolphthalein and then, drop by drop, run in dilute hydrochloric acid until the pink colour of the indicator is almost destroyed. To the liquid now add a considerable amount of common salt. A white precipitate will separate out. Filter this off through linen and save the filtrate which runs through.

The precipitate consists of soap and the following tests should be tried on it:

(a) Shake a little with distilled water, warming if necessary, and note that it dissolves and that the liquid froths considerably.

(b) Dissolve a little in water and add a dilute mineral acid. Note the precipitation of a white fatty substance which can be redissolved if the solution is again made alkaline with caustic soda.

(c) Dissolve a little in water and add a dilute mineral acid which will cause the precipitation of a white fatty substance. Add ether and shake up well. Note that the precipitate is soluble in ether and that, if the ether be poured off and evaporated in a dish on the water bath, the fatty substance will remain and probably solidify on cooling.

(d) Dissolve a little in water in two test-tubes and to one add calcium chloride solution while to the other magnesium chloride solution is added. Note the curdy white precipitate in both cases. (See note a.)

2. Exactly neutralise the filtrate separated from the soap in Expt. 1 and evaporate to as near dryness as possible on a water bath. Mix the residue with a small quantity of alcohol, stir well and filter. Again evaporate the filtrate on a water bath. Test the residue for glycerine by heating a small quantity with potassium hydrogen sulphate in a test-tube when the smell of acrolein will be obtained. (See note b.)

3. Try the following tests on lard:

(a) Shake a little with water and note that it does not dissolve. Heat and note that while the lard melts it is still insoluble.

(b) Repeat using alcohol instead of water as a solvent and note that in this case a small quantity of the lard dissolves on heating.

(c) Repeat using ether as the solvent and note that the lard is readily soluble even in the cold. Pour a few drops of the ether on to filter paper. The ether will evaporate leaving an oily stain.

4. Take a piece of manufacturers' soap and try the tests (a), (b), (c) and (d) described in Expt. 1 on it.

5. Boil up a small quantity of paraffin wax, cylinder oil or paraffin oil with caustic soda solution in a small flask. Note that even on prolonged boiling the oily globules do not disappear and that if the aqueous solution be poured off and acidified, no precipitate is caused as in the case of the lard in Expt. 1. (See note c)

NOTES

General note. The vegetable and animal oils and fats are esters, in which the alcohol is glycerine $C_3H_5(OH)_3$ and the acid is one or more of the fatty acids mentioned in Chap. VIII. The fatty acids most generally found are palmitic acid, stearic acid and oleic acid. Since glycerine contains three —OH groups it will be capable of combining with three molecules of a fatty acid. Thus, with palmitic acid, the reaction could be represented by the following equation:

$$C_3H_5(OH)_3 + 3C_{15}H_{31}.COOH = C_3H_5(O.CO.C_{15}H_{31})_3 + 3H_2O$$
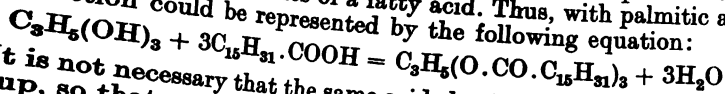
It is not necessary that the same acid should unite with each —OH group, so that a compound like

$$C_3H_5(O.CO.C_{15}H_{31})_2 (O.CO.C_{17}H_{35}),$$
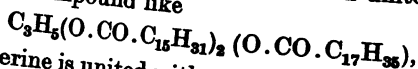
where the glycerine is united with two molecules of palmitic acid and one molecule of stearic acid, is quite possible. The compounds of these acids with glycerine are termed glycerides and special names are given such as tri-palmitin, tri-stearin, according to the acid present.

All the fats and oils of this nature are hydrolysed when heated with caustic soda with the production of the sodium salt of the acid and free glycerine. With the glyceride tri-palmitin the following would be the reaction:

$$C_3H_5(O.CO.C_{15}H_{31})_3 + 3NaOH = 3C_{15}H_{31}.COONa + C_3H_5(OH)_3$$
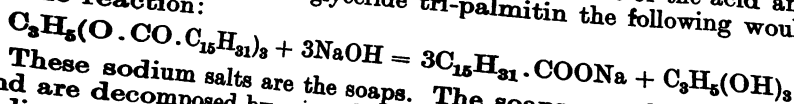
These sodium salts are the soaps. The soaps are soluble in water and are decomposed by mineral acids giving free fatty acids and the sodium salt of the mineral acid:

$$C_{15}H_{31}.COONa + HCl = C_{15}H_{31}.COOH + NaCl$$
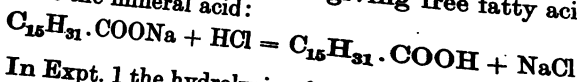
Note a. In Expt. 1 the hydrolysis of a fat is carried out as described in the general note. After the hydrolysis is complete, hydrochloric acid is added to neutralise the excess of soda and then common salt to "salt out" the soap, the latter being insoluble in salt solution although soluble in water. On filtering the soap remains on the filter but the glycerine passes through with the filtrate.

Expt. 1 (*b*) and (*c*). The precipitate consists of fatty acids from the soaps. The reaction has been shown at the end of the general note. These fatty acids are soluble in ether and can be extracted by this solvent.

Expt. 1 (*d*). The precipitates are the calcium and magnesium salts of the fatty acids, these salts being insoluble in water. This reaction takes place whenever soap is used with "hard" water.

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Note b. The glycerine from the original fat remains behind in the residue in the dish on evaporation. The alcohol extracts the glycerine, leaving the inorganic salts behind and on evaporating off the alcohol the glycerine remains.

Note c. It must be borne in mind that the mineral oils and waxes, paraffin oil, most lubricating oils, paraffin wax, etc., are not glycerides but hydrocarbons and are therefore not attacked by the most powerful hydrolysing agents.

XII

AMIDES

1. Acetamide, $CH_3.CONH_2$. Melt 30–50 grs. of ammonium acetate in a basin and when melted pour into a small retort, fitted with a thermometer and a long tube, slipped over the outlet tube of the flask, to act as a condenser. Heat gently and collect the distillate in two small beakers, the first to contain all the distillate up to 180° C and the second the distillate passing over between 180° C. and 220° C. The first distillate can be thrown away but the second is cooled and allowed to stand for a short time when it will partially solidify, and form a pasty mass. This mass is either filtered on a Buchner funnel or pressed out on a porous tile. The dry solid is acetamide. (See general note.)

2. Note the general appearance and odour of the acetamide and try the following tests on it:

(a) Dissolve a small quantity in water, add caustic soda solution and boil. Note the evolution of ammonia.

(b) Dissolve a small quantity in water, add dilute sulphuric acid and boil vigorously. Note that the steam smells of acetic acid and is acid to litmus paper.

(c) To a solution of acetamide add a small quantity of sodium nitrite solution and then a little very dilute hydrochloric acid. Note the effervesence caused by the evolution of nitrogen gas. (See note a.)

3. To a little ethyl oxalate in a test-tube add a few c.c. of strong ammonia solution. Filter off the white solid precipitated and wash it while on the filter paper with a little water. Try the following experiment on it:

(a) Boil a little with caustic soda and note that ammonia is given off and that the substance gradually dissolves. Make the solution in the test-tube acid with acetic acid and add calcium chloride solution. Note the white precipitate of calcium oxalate. (See general note and note b.)

4. Examine a specimen of urea and try the following experiments on it:

(a) By shaking up in turn with water, alcohol and ether show that urea is soluble in the first two solvents but not in ether.

(b) Boil a little with caustic soda solution and note that ammonia is evolved.

(c) To a solution of a little urea in water add sodium nitrite solution and then a little very dilute hydrochloric acid. Note the effervesence due to the evolution of nitrogen and carbon dioxide.

(d) To a solution of urea add a little alkaline hypobromite solution. Note the effervesence due to the evolution of nitrogen.

(e) To a small quantity of a strong solution of urea add a few c.c. of strong nitric acid. Note the white precipitate of urea nitrate. (See note c.)

NOTES

General note. The amides are a group of bodies characterised by containing the group —$CONH_2$. They can be considered as being formed from acids by the replacement of an —OH group by the —NH_2 group. Thus if an organic carboxylic acid is written $R.COOH$ and the terminal —OH group of this compound is replaced by —NH_2, we get $R.CONH_2$, which is the general formula for all amides of this group of acids. In carbonic acid there are two —OH groups, as shown when the formula for this acid is written $O:C.(OH)_2$. Here both —OH groups are replaceable by —NH_2 groups and there results $O:C.(NH_2)_2$, urea.

Two very general methods of preparing these substances are in use and these methods also indicate that the constitution of the amides is that given above. The first method is that of strongly heating the ammonium salts of the carboxylic acids. Take the case of the preparation of acetamide. Here ammonium acetate is heated, a molecule of water is eliminated and acetamide, $CH_3.CONH_2$, results. The reaction is as follows:

$$CH_3.COONH_4 = CH_3.CONH_2 + H_2O$$

The second general method of preparation is by acting on an ester with ammonia. Here alcohol is eliminated and the amide results. Taking the case of the preparation of oxamide, ammonia is added to ethyl oxalate and oxamide is formed. The reaction is:

$$(COOC_2H_5)_2 + 2NH_3 = (CONH_2)_2 + 2C_2H_5OH$$
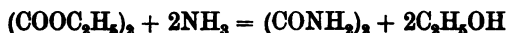
The great majority of the amides are white crystalline bodies, soluble in water, hydrolysed by boiling either with alkalis or mineral acids and decomposed, with the liberation of nitrogen, both by nitrous acid and alkaline hypobromites.

Note a. Expt. 2 (*a*). The reaction taking place is as follows:

$$CH_3.CONH_2 + NaOH = CH_3.COONa + NH_3$$
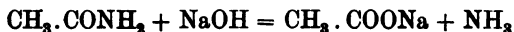
Expt. 2 (*b*). The following reaction takes place:

$$2CH_3.CONH_2 + H_2SO_4 = 2CH_3.COOH + (NH_4)_2SO_4$$

Expt. 2 (*c*). The reaction taking place is:

$$CH_3.CONH_2 + HNO_2 = CH_3.COOH + H_2O + N_2$$
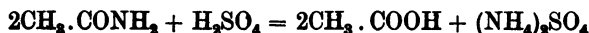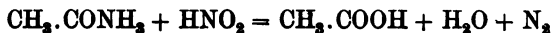
The nitrous acid, HNO_2, is formed by the interaction of the sodium nitrite, $NaNO_2$, and the hydrochloric acid.

Note b. Expt. 3 (*a*). When the oxamide is boiled with caustic soda the following reaction takes place:

$$(CONH_2)_2 + 2NaOH = (COONa)_2 + 2NH_3$$
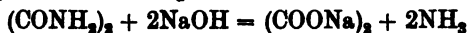
that is, sodium oxalate and ammonia are formed.

Note c. In Expt. 4 the following reactions take place:

(*b*) Urea boiled with caustic soda gives ammonia and sodium carbonate. $CO(NH_2)_2 + 2NaOH = Na_2CO_3 + 2NH_3$

(*c*) Urea treated with nitrous acid gives carbon dioxide, water and nitrogen.

$$CO(NH_2)_2 + 2HNO_2 = CO_2 + H_2O + 2N_2$$
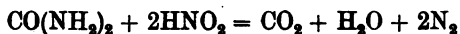
(*d*) Urea with alkaline hypobromite solution gives sodium carbonate, sodium bromide, water and nitrogen.

$$CO(NH_2)_2 + 3NaOBr + 2NaOH = Na_2CO_3 + 3NaBr + 2H_2O + N_2$$
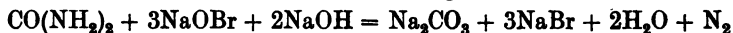
(*e*) Urea shows slightly basic properties, that is, it gives salts with strong acids. With nitric acid, it forms urea nitrate, $CO(NH_2)_2 . HNO_3$, a salt sparingly soluble in water.

XIII

AMINES

1. Examine the specimens of methylamine and trimethylamine provided and note their characteristic odours. (See general note.)

2. Examine the sample of aniline and try the following experiments on it:

(a) Shake up a small quantity with water and note that it is insoluble. Add dilute hydrochloric acid when the amine will dissolve. To this acid solution add caustic soda solution until alkaline and note the reprecipitation of the oily aniline.

(b) To a few drops of strong sulphuric acid in a porcelain dish add one or two drops of aniline. Note the formation of a white solid, aniline sulphate.

(c) To a solution of aniline in dilute hydrochloric acid add a solution of sodium nitrite and warm. Note that an effervesence takes place, due to the evolution of nitrogen, and the odour of phenol is observed.

(d) To a solution of a few drops of aniline in hydrochloric acid add a few drops of stannous chloride solution. Note the formation of a white precipitate, a compound of aniline hydrochloride and stannous chloride. (See general note and note a.)

ALKALOIDS

3. Examine the specimen of quinine and try the following tests on it. (See general note.)

(a) Shake up a very small quantity with water and note that it is insoluble. To the solution add a small quantity of dilute hydrochloric or sulphuric acid and note that the quinine dissolves. To this solution add caustic soda and note the reprecipitation of quinine. Now add ether to the solution and shake vigorously. Note that the quinine dissolves in the ether.

(b) Dissolve a little quinine sulphate in water and divide the solution into three parts. To the first add a solution of picric acid, to the second a solution of phosphotungstic acid and to the third a solution of mercuric iodide in potassium iodide. Note that in each case a precipitate is produced. (See note a.)

URIC ACID

4. Examine the specimen of uric acid, note its general appearance and try the following tests on it:

(a) Shake up with water and note that it is almost insoluble. Warm the solution and note that some, if not all, will dissolve.

(b) To a small quantity of uric acid add dilute caustic soda solution and warm. Note that the uric acid dissolves and that on acidifying with hydrochloric acid and cooling the uric acid will separate out, if the solution be sufficiently concentrated.

(c) To a small quantity of uric acid in a porcelain dish add a few drops of strong nitric acid. Heat gently over a flame until most of the nitric acid has evaporated and the yellow residue begins to turn red. Allow to cool and then touch the yellow residue with a drop of ammonia on a glass rod. Note the purple-red which is produced. (Murexide test.)

(d) Separate a specimen of uric acid from guano in the following way. Boil a small quantity of guano with caustic soda solution until all smell of ammonia has disappeared. Filter, cool the solution and acidify it with nitric acid. A precipitate will be formed, which is impure uric acid. Try the murexide test, described in (c) above, on it. (See general note, Uric Acid.)

NOTES

AMINES

General note. The amines are a class of bodies characterised by having basic properties and which can be considered as being derived from ammonia by the replacement of hydrogen by hydrocarbon radicles. Thus by the replacement of one atom of hydrogen in ammonia, NH_3, by the radicle methyl, the compound methylamine, $CH_3.NH_2$, would result. If two hydrogen atoms were replaced by methyl groups, the compound $(CH_3)_2.NH$, dimethylamine, would result, while the replacement of three hydrogen atoms in the same way would give trimethylamine $(CH_3)_3.N$. The amines are known as primary, secondary or tertiary amines, according as one, two or three hydrocarbon radicles have entered the ammonia molecule. Methylamine, $CH_3.NH_2$, and aniline, $C_6H_5.NH_2$, are amongst the commonest primary amines, whilst trimethylamine, $(CH_3)_3.N$, is a common tertiary amine.

All the amines are basic bodies, forming salts with acids and behaving in many respects like ammonia itself.

Note a. Expt. 2 (*a*). The aniline dissolves in hydrochloric acid with the formation of aniline hydrochloride, $C_6H_5.NH_2.HCl$. The caustic soda decomposes this reprecipitating aniline, $C_6H_5.NH_2$.

Expt. 2 (*b*). The aniline unites with the sulphuric acid giving aniline sulphate, $(C_6H_5.NH_2)_2.H_2SO_4$.

Expt. 2 (*c*). The aniline is decomposed by the nitrous acid with the liberation of nitrogen and the formation of phenol.

$$C_6H_5.NH_2 + HNO_2 = C_6H_5.OH + H_2O + N_2$$
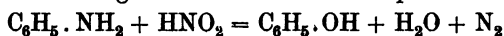
This is a general reaction for all primary amines.

Expt. 2 (*d*). The amines, like ammonia, frequently give complex compounds with metallic salts. In this case the compound $(C_6H_5.NH_2.HCl)_2.SnCl_2$ is formed. The most frequently used of these metallic salts are the platino-chlorides of the general type $(R.NH_2.HCl)_2.PtCl_4$, which are frequently used for the separation and purification of amines.

ALKALOIDS

General note. The alkaloids are a group of complex nitrogenous and basic bodies of frequent occurrence in plants. The term is somewhat loosely used and the various alkaloids do not conform to any particular chemical structure. They are, however, nearly all insoluble in water, soluble in ether or chloroform, sufficiently basic to form salts with

acids and nearly all of them give some important physiological re-
action. They include such important substances as

quinine $C_{20}H_{24}N_2O_2$, strychnine $C_{21}H_{22}N_2O_2$, nicotine $C_{10}H_{14}N_2$, etc.

Note a. Expt. 3 (*a*). The quinine dissolves in, say, sulphuric acid
with the formation of quinine sulphate, $(C_{20}H_{24}N_2O_2)_2 . H_2SO_4$, which
is soluble in water. Caustic soda decomposes this with the precipita-
tion of quinine base.

Expt. 3 (*b*). The reagents used in this experiment are very common
precipitating agents for alkaloids and, indeed, for many complex
nitrogenous substances. They frequently serve as a means of separat-
ing these substances from mixtures.

URIC ACID

General note. Uric acid is the commonest of a class of bodies
known as the purines, all of which have the skeleton structure

$$
\begin{array}{ccc}
N & - & C \\
| & & | \\
C & & C - N \\
| & & \parallel \quad \diagdown \\
& & \quad\quad\quad C \\
N & - & C - N \diagup \\
\end{array}
$$
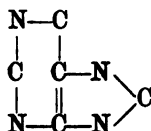
Uric acid itself has the constitutional formula

$$
\begin{array}{ccc}
HN & - & C = O \\
| & & | \\
O = C & & C - NH \\
| & & \parallel \quad\quad \diagdown \\
& & \quad\quad\quad\quad C = O \\
HN & - & C - NH \diagup \\
\end{array}
$$
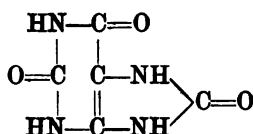
It is an important substance physiologically and occurs in urine.
It is a feebly acid substance forming salts with bases, these salts
being much more soluble than the acid itself and it can be precipitated
from solutions of these salts by the addition of acids. Birds' excreta
contains large quantities of uric acid and so guano serves as a con-
venient source from which to prepare this compound

XIV

CARBOHYDRATES

Monosaccharides, $C_6H_{12}O_6$ and $C_5H_{10}O_5$.

1. Dextrose, $C_6H_{12}O_6$. Known also as glucose or grape sugar. Examine a specimen of dextrose and try the following experiments with it:

(a) Heat a small quantity in a dry test-tube. The glucose melts and gradually blackens, while water is given off. The smell of burnt sugar is observed and finally only a charred mass remains.

(b) Heat a small quantity with caustic soda solution and note that the liquid becomes dark brown while the smell of burnt sugar is observed. The smell becomes more distinct on acidifying.

(c) Heated with strong sulphuric acid pure glucose does not blacken but commercial samples will frequently do so.

(d) To some silver nitrate solution in a test-tube add ammonia solution, drop by drop, until the precipitate which first forms just re-dissolves. To this reagent add a small quantity of glucose solution and heat up the mixture by standing the test-tube in a water bath. Note the silver mirror formed on the sides of the tube. The test-tube must be scrupulously clean before this test is tried.

(e) To a small quantity of dextrose solution add an excess of Fehling's solution. Note that no change takes place in the cold but that on warming a red precipitate of cuprous oxide is thrown down.

(f) Heat together a mixture of dextrose solution and Barfoed's reagent (an acetic acid solution of copper acetate) and note that a red precipitate of copper oxide is thrown down.

(g) To a little dextrose solution add a mixture of phenylhydrazine and acetic acid (about 10 drops of each). Heat in a water bath for about half an hour. When a yellow precipitate begins to be deposited take out of the water bath and allow to cool. A yellow crystalline precipitate of phenyl-glucosazone will now separate out. Place a few of these crystals on a microscope slide and examine under a microscope. They will be seen to have a characteristic feathery appearance.

(h) Make a solution of glucose and with it fill one of the polarimeter tubes. Place the tube in the polarimeter and note that the solution is dextro-rotatory. (See note a.)

2. Levulose, $C_6H_{12}O_6$. Known also as fructose or fruit sugar. Examine the specimen of levulose and try on it all the tests given above for dextrose. Note the great similarity of behaviour of the two sugars except in the polarimeter, where levulose is laevo-rotatory. Note that levulose gives the same osazone with phenylhydrazine as dextrose. (See note b.)

3. Pentoses (Arabinose, Xylose), $C_5H_{10}O_5$. Prepare a solution of pentose sugars by boiling a little gum arabic with equal quantities of water and hydrochloric acid for 10–15 minutes. On the resulting liquid try the following tests:

(a) Neutralise with caustic soda, add excess of Fehling's solution and heat. Note the reduction to red cuprous oxide.

(b) To equal quantities of hydrochloric acid and water add a very small quantity of phloroglucinol. To this mixture add some of the hydrolysed gum solution prepared above. Warm in a water bath. Note that the colour becomes cherry red and a precipitate is formed. Allow to cool and shake with amyl alcohol. This will dissolve the precipitate giving a red solution.

4. Try also the following test for pentoses. In a small distilling flask place a few grams of gum arabic and diluted hydrochloric acid (40 c.c. strong acid and 60 c.c. water). Support the flask in a clamp and slip over the outlet tube a wider tube to serve as a condenser. Boil the liquid in the flask and test the drops of liquid coming from the condenser, by allowing them to drop on to a small piece of filter paper moistened with aniline acetate solution. Note the bright red stain produced on the test paper. This reaction is common to all pentoses. (See note c.)

NOTES

General note. Carbohydrates. These are a class of bodies containing carbon, hydrogen and oxygen, in which the hydrogen and oxygen are present in the same proportion as in water, namely two atoms of hydrogen to one atom of oxygen. (Compare the formulae given below.) This class contains all the sugars, starches, dextrins and celluloses, besides lesser known substances such as gums, mucilages, pectins, etc. They differ considerably in physical properties, the simpler ones being crystalline and soluble in water while the more complex members are amorphous and insoluble. Their relationship is shown by the fact that the more complex members, on hydrolysis with acid, break down, yielding one or more of the simple sugars. The following table gives a simple classification of them:

Sugars	Monosaccharides	Pentoses $(C_5H_{10}O_5)$.	Arabinose, Xylose, etc.
		Hexoses $(C_6H_{12}O_6)$.	Dextrose, Levulose.
	Disaccharides	$C_{12}H_{22}O_{11}$.	Cane sugar, Maltose, Lactose.
Non-sugars	Polysaccharides	$(C_6H_{10}O_5)_n$.	Starches, Dextrins, Celluloses.
		$(C_5H_8O_4)_n$.	Gums, Mucilages, Pectins.

The constitution of the simpler carbohydrates helps greatly to explain their reactions. They are all polyhydroxy alcohols and the simplest members are, in addition, aldehydes or ketones. Thus the formula for dextrose can be written $CH_2OH.(CH.OH)_4.CHO$, while that for levulose can be written $CH_2OH(CH.OH)_3.CO.CH_2OH$. The pentoses also have similar constitutions and both arabinose and xylose can be written $CH_2OH.(CH.OH)_3.CHO$.

Note a. Expt. 1 (*a*). This reaction has already been explained when dealing with aldehydes. The silver oxide, Ag_2O, in solution is reduced to metallic silver, while the aldehyde, in this case the dextrose, is oxidised to the corresponding acid.

Expt. 1 (*c*). In this case the aldehyde, dextrose, reduces the cupric oxide, CuO, to cuprous oxide, Cu_2O.

Expt. 1 (*f*). Here again the copper compound is reduced to Cu_2O. This experiment is important as the reaction serves to distinguish glucose and levulose from lactose and maltose. All four sugars reduce Fehling's solution but only the first two reduce Barfoed's solution.

Expt. 1 (*g*). This reaction is a very important one. It takes place in two stages. At first the phenylhydrazine forms the simple hydrazone with the dextrose, the reaction being represented by the following equation:

$$CH_2OH.(CH.OH)_4.CHO + C_6H_5NH.NH_2$$
$$= CH_2OH.(CH.OH)_4.CH: N.NH.C_6H_5 + H_2O$$
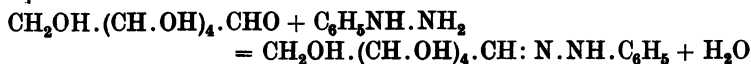
The $=CH.OH$ group next to the original aldehyde group is next oxidised to a $=CO$ group by excess of phenylhydrazine giving the body $CH_2OH.(CH.OH)_3.CO.CH: N.NH.C_6H_5$ and this then reacts like all ketones with another molecule of the hydrazine to give the final product

$$CH_2.OH.(CH.OH)_3.C(: N.NH.C_6H_5).CH: (N.NH.C_6H_5)$$

a body known as an osazone, in this case glucosazone.

Levulose acts in a similar way, the characteristic ketone group in this sugar reacting first with phenylhydrazine, then the terminal —CH_2OH group of the levulose is oxidised to a —CHO group and this then reacts with another molecule of the hydrazine to give the same osazone as that from dextrose. The osazones are most important in the identification of the sugars, since they frequently give characteristic crystals and when pure very definite melting points, while the varying times taken before they are precipitated are often a help also in saying what sugar is present.

Note b. The reactions of levulose are practically identical with those of dextrose and the most certain way of distinguishing between them is by the polarimeter.

Note c. Arabinose and xylose are aldehydes, similar in structure to glucose and give the characteristic reduction with Fehling's solution.

The pentoses have the characteristic reaction that when heated with hydrochloric acid they lose three molecules of water and give the aldehyde, furfural, $C_4H_3O.CHO$. It is this body which distils in the steam and gives the red coloration with aniline acetate.

XV

CARBOHYDRATES (cont.)

Disaccharides, $C_{12}H_{22}O_{11}$.

1. Cane sugar, called also sucrose and saccharose. Examine the sample of cane sugar and carry out the following tests with it:

(a) Boil with caustic soda solution and note that no change takes place.

(b) Warm with ammoniacal silver nitrate solution and note no reduction takes place.

(c) Warm with Fehling's solution and note that no reduction takes place.

(d) Heat with Barfoed's solution and again note that there is no reduction.

(e) Warm with acetic acid and phenylhydrazine, as described for dextrose, and note there is no osazone formation.

(f) Make a solution in water and examine in the polarimeter and note the solution is dextro-rotatory.

(g) Mix with concentrated sulphuric acid and note that charring takes place even in the cold but that this is much more pronounced if the solution is warmed, much gas being evolved and a mass of porous carbon left behind.

(h) To a solution of cane sugar add a few c.c. of hydrochloric acid and boil for a few minutes. Neutralise the excess of acid with caustic soda and try tests (b), (c), (d) and (e) above on the solution. Note that these tests, instead of giving negative results, now give positive results.

Note also that the solution after boiling with the acid is laevo-rotatory instead of dextro-rotatory. (See note a.)

2. Lactose, also called milk sugar. Examine a sample of lactose, noting that it is only moderately sweet to the taste, and try the following experiments on it:

(a) Try tests (b), (c), (d), (e), (f), and (g), as described for dextrose on small quantities of lactose. Note that with caustic soda solution it goes brown, that it blackens when heated with strong sulphuric acid, that it reduces ammoniacal silver nitrate solution and Fehling's solution but not Barfoed's solution and that no osazone is precipitated while the solution is hot and only separates on the solution being cooled down. Examine the crystalline form of the osazone under the microscope. It consists chiefly of nodules of fine needles and is very

different from the osazone of dextrose and levulose. It should be noted that much longer heating may be required for the formation of this osazone than for that of dextrose.

(b) Make a solution of lactose in water in a small beaker. From this solution fill a polarimeter tube and read the amount of dextro-rotation as given by the polarimeter. Pour the solution from the tube back into the beaker and make a mark on the beaker showing the level at which the liquid stands. Add a few c.c. of hydrochloric acid to the solution and boil for some minutes. Make up the liquid in the beaker to the mark with distilled water and again take a reading in the polarimeter tube. Note that the amount of dextro-rotation has now increased. (See note b.)

(c) About 1–2 grs. of lactose or 10–15 c.c. of the solution to be tested for lactose are placed in a porcelain dish and 5–10 c.c. of strong nitric acid are added. The dish is placed on a water bath and the solution evaporated until all red fumes cease to be given off. The solution is then diluted with about 50 c.c. of water and allowed to stand a short time when a white granular precipitate of mucic acid separates out. (See note c.)

3. Maltose. Examine a sample of maltose and try the following experiments with it:

(a) Try tests (b), (c), (d), (e), (f) and (g) as described for dextrose on a solution of maltose. Note that the results are practically the same as for lactose except that the osazone again has a distinctive appearance under the microscope, the maltosazone occurring in rosettes of stout needles. Much longer heating is required for the production of this osazone than for that of dextrose.

(b) Repeat Expt. 2 (b), as described for lactose, using maltose. Note that hydrolysis with hydrochloric acid decreases the degree of dextro-rotation of the solution. (See note b.)

NOTES

Note a. Cane sugar does not contain any free aldehyde or ketone groups in its molecule and does not therefore give any of the characteristic reducing actions with such reagents as Fehling's solution, etc., such as are shown by dextrose and levulose. By the action of mineral acids however, cane sugar is hydrolysed into a mixture of dextrose and levulose, the reaction being represented by the following equation.

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$$
Cane Sugar + Water = Dextrose + Levulose
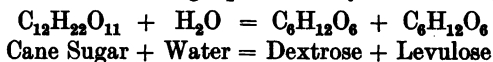
The resulting solution will therefore give all the characteristic reactions of these two sugars.

In the hydrolysed solution, of the two sugars present, levulose is more strongly laevo-rotatory than dextrose is dextro-rotatory, so that the solution, as a whole, is laevo-rotatory. Because the rotation is changed from dextro to laevo on hydrolysis, this process is sometimes spoken of as "inversion" and the mixture of the two sugars is referred to as "invert sugar."

Note b. Lactose and maltose contain free aldehyde groups in their molecules and therefore show the ordinary reducing reactions with alkaline solutions of metallic oxides such as Fehling's solution. They do not however reduce the acid Barfoed's solution.

On hydrolysis with acid they break up into monosaccharides in the following way:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$$
Lactose + Water = Dextrose + Galactose

$$C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$$
Maltose + Water = Dextrose
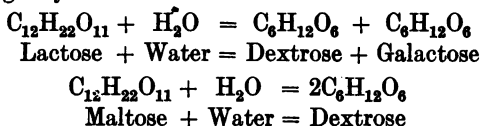
The mixture of dextrose and galactose is more strongly dextrorotatory than the original lactose and so the rotation is increased while the two molecules of dextrose from the maltose are less strongly dextro-rotatory than the original maltose and the rotation is therefore diminished.

Note c. The formation of mucic acid from lactose is a very good method of distinguishing this sugar from the others dealt with. The lactose is first hydrolysed by the acid to a mixture of dextrose and galactose and these sugars are then oxidised by the nitric acid. The oxidation product of the galactose, mucic acid,

$$HOOC.(CH_2OH)_4.COOH,$$

is almost insoluble in water and separates out therefore on dilution.

XVI

CARBOHYDRATES (cont.)

Polysaccharides, $(C_6H_{10}O_5)_n$.

1. Starch. Examine a sample of starch and carry out the following experiments on it:

(a) Shake up a little with cold water and note that it is insoluble. Warm the mixture and note that a viscous opalescent liquid is produced giving the impression that the starch has gone into solution.

(b) Dilute a little of the liquid obtained in (a), add Fehling's solution and warm. Note that no reduction takes place.

(c) To a little of the solution obtained in (a), add a small quantity of hydrochloric acid and boil for a few moments. Cool, neutralise the acid with caustic soda solution, add Fehling's solution and warm. Note the reduction of the Fehling's solution.

(d) To small quantities of the solution made in (a) add alcohol and basic lead acetate solution. Note that in both cases the starch is precipitated from solution.

(e) Dilute largely a small quantity of the solution made in (a). Add a small quantity of iodine solution and note the deep blue colour produced. Warm the solution and the colour will disappear while it will reappear again on cooling.

(f) Examine starch grains from various sources (wheat, potato, etc.) under the microscope and note their characteristic shapes. (See note a.)

2. Dextrin. Examine a sample of dextrin and make a solution of a small quantity in water. Try all the experiments described above for starch on this solution and note the results. They should differ from those obtained for starch in the following particulars: dextrin gives a clear solution with water instead of a viscous colloidal solution, it gives a red coloration with iodine instead of a deep blue one, it is not precipitated by basic lead acetate. (Some samples of dextrin may reduce Fehling's solution slightly but this reduction will be very greatly increased after hydrolysis with acid.) (See note b.)

3. Cellulose. Take cotton wool as a typical cellulose and try the following experiments on it:

(a) Heat small quantities with water, dilute acids and dilute alkalis and note that it is insoluble in all these reagents.

(b) Treat a small quantity of cotton wool with strong sulphuric acid and note that it dissolves. Allow to stand by a short time and

then dilute with water. Neutralise the acid present with caustic soda, add Fehling's solution and boil. Note that the Fehling's solution is reduced.

(c) Treat a small quantity of cotton wool with Schweitzer's reagent (an ammoniacal solution of copper oxide) and note that the cellulose dissolves and is re-precipitated as a powder on the addition of acid. (See note c.)

4. Gums and Mucilages. Examine samples of gum arabic and linseed mucilage as being typical of these classes of bodies. Note that they dissolve in water to give colloidal solutions and that these solutions do not reduce Fehling's solution before boiling with dilute acids but do so after that treatment.

Refer to Expt. 3, Chap. XIV, showing that pentose sugars are formed amongst other products when these substances are hydrolysed.

Glycogen and Inulin. Examine small quantities of these substances and, if available in sufficient quantities, carry out on them the same experiments as those described for starch.

GLUCOSIDES

Amygdalin. Examine the specimen of amygdalin and carry out the following tests on it:

(a) Dissolve a very small quantity in water and show that it does not reduce Fehling's solution.

(b) Hydrolyse a very small quantity by boiling with dilute sulphuric acid in a test-tube. Note the smell of hydrocyanic acid and test for its presence in the vapours from the tube by holding in them a glass rod moistened with silver nitrate solution. The silver nitrate will become opalescent owing to the formation of a precipitate of silver cyanide.

Test the solution in the test-tube with Fehling's solution and note that this will be reduced by the benzaldehyde liberated. (See note a.)

NOTES

General note. The value of the factor "n" in the formula $(C_6H_{10}O_5)_n$ is unknown but it is probably large. Its value must, of course, vary from one polysaccharide to another being smaller in the case of dextrin, say, than in the case of starch.

Note a. Expt. 1 (a). The term "starch solution" is frequently used for the liquid obtained by treating starch with hot water but it must be remembered that the starch is not in true solution but only forms a colloidal suspension.

Expt. 1 (c). The starch is hydrolysed by boiling with acids to give glucose which then reduces Fehling's solution. The hydrolysis can be represented by the following equation:

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$$
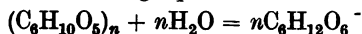
Expt. 1 (d). The precipitation of starch by these two reagents is somewhat important as it frequently gives a means of separating starch from sugars.

Expt. 1 (e). The composition of the blue compound of starch and iodine is not definitely known. It is most important to note the destruction of this blue colour by heat. In view of this, it is obviously impossible to test for starch with iodine in hot solutions.

Expt. 1 (f). The starch grains vary considerably in size and shape from different plant tissues. The appearance of the commoner ones should be committed to memory.

Note b. Dextrin is hydrolysed by dilute acids to glucose in a similar manner to starch. It therefore gives a strongly reducing action after treatment with dilute acids.

Note c. "Cellulose" is a term used somewhat loosely to describe the woody portion of plant tissues. The plant celluloses certainly contain more than one chemical individual but all these are characterised by certain general properties, such as their insolubility in water and all ordinary solvents and their comparative stability to chemical reagents. For the purpose in view there is no need to sub-divide the celluloses and cotton wool is taken as an example of the whole class.

Expt. 3 (b). The cotton wool is hydrolysed by treatment with sulphuric acid and the subsequent addition of water to give dextrose, and the hydrolysed solution consequently reduces Fehling's solution.

Note d. The glucosides are a group of compounds occurring in plant tissues and are characterised by breaking up on hydrolysis into glucose together with one or more other substances, frequently of an

aromatic nature. One of the best known glucosides is amygdalin, $C_{20}H_{27}NO_{11}$, which breaks up on hydrolysis into two molecules of glucose, one molecule of hydrocyanic acid and one molecule of benzaldehyde.

$$C_{20}H_{27}NO_{11} + 2H_2O = 2C_6H_{12}O_6 + C_6H_5.CHO + HCN$$
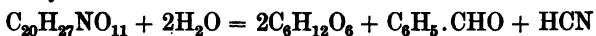
The glucosides occur only in small quantities in plant tissues but the possibility of them giving hydrolysis products of a highly poisonous nature, as in the case of the hydrocyanic acid from amygdalin, give these bodies considerable practical importance.

XVII

PROTEINS

1. Examine samples of egg-albumen, gelatine, casein, and horn and apply the following tests to them:

(a) Heat with dilute caustic soda solution and note that no ammonia is given off on boiling.

(b) Heat a little of each with soda-lime in a dry tube and note that in each case ammonia is given off in considerable quantity.

(c) Heat one or two of the above substances with a little metallic sodium as described in Expt. 6, Chap. I, and test the solutions obtained for nitrogen and sulphur as described therein.

(d) Mix a small quantity of casein with anhydrous sodium carbonate and potassium nitrate (equal parts of each) and ignite the whole mass strongly in a porcelain crucible. Cool and dissolve out the fused mass by boiling the crucible and its contents in water in a beaker. Test the solution obtained for phosphate by acidifying with strong nitric acid, adding ammonium molybdate and warming, when a yellow precipitate will result. (See note a.)

2. Heat a small quantity of egg-albumen solution, to which one drop of dilute acetic acid has been added, and note that the albumen is coagulated and precipitates as a flocculent precipitate. (See note b.)

3. To solutions of egg-albumen and gelatine add a large excess of alcohol and note that in both cases the protein is precipitated. (See note c.)

4. To a small quantity of wheat gluten add 70 per cent. alcohol and shake vigorously. Filter and evaporate the filtrate to dryness on a water bath. Note the residue of protein in the dish and apply to it some of the tests described in this chapter to prove its protein nature. (See note d.)

5. Repeat Expt. 4 but divide the solution in 70 per cent. alcohol into two portions. To one portion add a large excess of water and to the other a large excess of absolute alcohol. Note that in both cases the protein in solution is precipitated. (See note d.)

6. On a solution of egg-albumen or gelatine carry out the following tests:

(a) Add a few drops of mercuric chloride solution and note the heavy white precipitate formed.

(b) Add, to separate portions, lead acetate solution and basic lead acetate solution and note a white precipitate in each case.

7—2

(c) Add, drop by drop, a solution of copper sulphate. Note the bluish precipitate which will dissolve to a violet solution on adding caustic soda. (See note e.)

7. To a solution of egg-albumen or gelatine apply the following tests:

(a) Add a solution of picric acid and note the formation of a yellow precipitate.

(b) Add a solution of tannic acid and note the brownish precipitate formed.

(c) Add a solution of phosphotungstic acid and note the white precipitate formed. (See note f.)

8. (a) To a few c.c. of albumen solution add one c.c. of strong nitric acid. A white precipitate will be formed. Boil for a few minutes when the precipitate will partly dissolve and the remainder will go yellow. Cool the solution and add strong ammonia till alkaline. The yellow colour will be turned to orange. (Xanthoproteic reaction.)

(b) Repeat the experiment using a few small pieces of horn instead of albumen solution. Note that the colour changes are the same on the surface of the pieces of horn although the substance does not pass into solution.

(c) To a few c.c. of albumen solution add half its volume of Millon's reagent. A white precipitate is formed which, on gently warming, turns brick red in colour or dissolves to form a red solution. (Millon's reaction.)

(d) To a few c.c. of the albumen solution add one c.c. of 40 per cent. caustic soda solution. Then add one drop of 1 per cent. copper sulphate solution. A violet-pink colour is produced. (Biuret reaction.)

(e) To a few c.c. of the albumen solution add an equal bulk of "reduced oxalic acid" solution and then carefully, down the side of the test-tube, a quantity of strong sulphuric acid. A purple ring forms where the two liquids meet. (Glyoxylic reaction.)

(f) Add a drop of lead acetate solution to a solution of egg-albumen and then caustic soda sufficient to redissolve the precipitate which is first formed. Boil the mixture and note the brown or black coloration which is formed. (Sulphur reaction.) (See note g.)

NOTES

General note. The proteins are a group of bodies containing approximately carbon 53 per cent., hydrogen 7 per cent., oxygen 22 per cent., nitrogen 16 per cent., and small and variable amounts of sulphur, phosphorus and ash. They vary very considerably in physical pro perties and to some extent in chemical properties but all have the following properties in common; they are non-crystalline bodies containing about 16 per cent. nitrogen, this nitrogen is not liberated by boiling with caustic soda but is liberated as ammonia on heating with soda-lime and they all, as a result of prolonged hydrolysis with mineral acids, break down giving various amino-acids (see next chapter).

Note a. The reactions involved in these tests have been explained in Chap. I.

Note b. This property of coagulating on heating is not a general property of soluble proteins, but is peculiar to a restricted number, the albumins being the most important of those behaving in this manner.

Note c. This property of being precipitated by alcohol is very important in connection with practical work on proteins since it frequently presents us a means of separating these bodies from other substances.

Note d. The protein of wheat, known as gliadin, and that of maize, known as zein, are two of the very few proteins showing this solubility in 70 per cent. alcohol. The majority of proteins are quite insoluble in this solvent. Even in these two cases stronger alcohol causes precipitation.

Note e. Many of the salts of heavy metals cause precipitation of proteins and the three tests given are merely chosen as examples. The property is sometimes made use of in technical analysis, e.g. the removal of protein from a solution in which it is required to estimate the percentage of sugar.

Note f. These reactions are examples of the use of what are frequently called the alkaloidal reagents in the precipitation of proteins. They are reactions of great value when proteins are to be removed from solutions and the tannic acid reaction is of technical importance in the tanning of leather.

Note g. Expt. 8 (*a*). The xanthoproteic reaction is of great use in demonstrating the protein nature of a substance. The yellow colour is due to the action of the nitric acid on some aromatic group in the

protein, a nitro derivative of this being formed. These aromatic substances are phenyl-alanine, tyrosine and tryptophane. (See next chapter.)

Expt. 8 (c). Millon's reagent is made by dissolving mercury in nitric acid and subsequently diluting. It contains the nitrates of mercury, an excess of nitric acid and a little nitrous acid. The formation of a red colour with this reagent depends on the presence of the tyrosine group in the protein molecule. (See next chapter.)

Expt. 8 (d). The biuret reaction is given by most proteins. It is given by practically all compounds containing two —CO.NH— groups attached to one another, to the same nitrogen atom or to the same

carbon atom. The last-named grouping —CO.NH.$\overset{|}{\text{C}}$H.NH.CO—

occurs with great frequency in proteins which consequently give this colour reaction.

Expt. 8 (e). The "reduced oxalic acid" reagent contains glyoxylic acid, COOH.CHO, and the colour produced depends on the reaction of this substance with the tryptophane group in the protein molecule.

Expt. 8 (f). The brown or black colour is due to the formation òf lead sulphide owing to the separation of sulphur from the cystine unit of the protein by the action of the boiling alkali.

XVIII

PROTEINS (*cont.*)

1. Weigh out 10 grs. of gluten and place in a 100 c.c. round-bottomed flask. Add 30 c.c. of strong hydrochloric acid and warm until the gluten is dissolved. Cork the flask with a cork through which passes a long glass tube to serve as a reflux condenser. Place on a sand bath and boil for twelve hours. At the end of this time add about 3 grs. of animal charcoal, boil and filter, washing the residue on the filter paper with hot water. Evaporate the filtrate and washings to a bulk of about 20 c.c., transfer to a boiling tube, cool to 0° C. by standing in ice and saturate with hydrochloric acid gas. When the liquid is saturated, as shown by the escape of copious fumes from the boiling tube, seed the liquid with a crystal of glutaminic acid hydrochloride, cork the tube loosely and place in the ice chest. Examine after two days. If very few crystals have separated, cool for two or three hours in a freezing mixture of ice and salt and replace in the ice chest. Repeat this treatment if necessary. When further crystals cease to separate, add an equal volume of ice cold alcohol and filter off the crystals of glutaminic acid hydrochloride, using a Buchner funnel on the water-pump. Wash with a very little ice cold alcohol. Transfer the crystals to a weighed dish, dry in the water oven and weigh as glutaminic acid hydrochloride, $C_5H_9O_4.HCl$. Calculate the percentage of glutaminic acid in the gluten taken. Recrystallise your specimen of hydrochloride from the least possible quantity of hot water. (See general note.)

2. Weigh out accurately about 1 gr. of protein. Transfer to a small round-bottomed flask, add 20 c.c. of strong hydrochloric acid and boil for at least twelve hours on a sand bath, with a reflux condenser, as in Expt. 1. Wash out of the flask into an evaporating dish and evaporate to dryness on a water bath in a fume cupboard. Wash with hot water into a large round-bottomed flask, add an excess of a thin cream made with magnesia and water, and distil with steam into 35 c.c. of N/10 acid. Add methyl orange and titrate back with N/10 alkali. Calculate the percentage of nitrogen liberated as ammonia on hydrolysis. This percentage varies from one to five and is a measure of the proportion of amide groups, —$CONH_2$, present. (See note *a*.)

3. Compare roughly the amounts of tyrosine, tryptophane and cystine in gelatine and egg-albumen by trying Millon's reaction, the glyoxylic reaction, and the sulphur test, on the two proteins and noting the depth of the different colours, if any, produced. (For details of these tests see preceding chapter.)
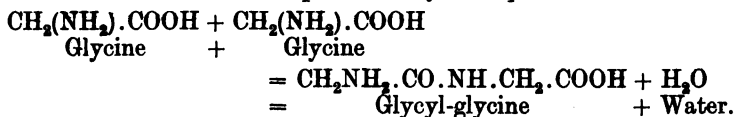
NOTES

General note. The proteins are a most important group of chemical compounds since they occur in all living cells and are an essential constituent of food. They are built up by the condensation of molecules of amino-acids in a way roughly indicated below and these amino-acids can be considered as the units of the protein molecule. At least twenty different amino-acids are known and of these the following are quoted as being fairly typical of different groups of these bodies:
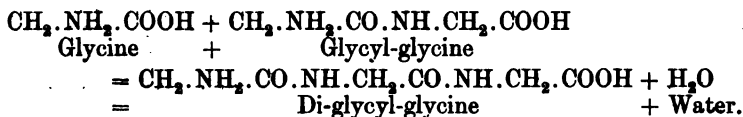
1. Glycine, $NH_2.CH_2.COOH$.
2. Alanine, $CH_3.CH(NH_2).COOH$.
3. Phenyl-alanine, $C_6H_5.CH_2.CH(NH_2).COOH$.
4. Tyrosine, $C_6H_4 \diagup^{OH}_{\diagdown CH_2.CH(NH_2).COOH}$
5. Glutaminic Acid, $HOOC.CH_2.CH_2.CH(NH_2).COOH$.
6. Lysine, $CH_2.NH_2.CH_2.CH_2.CH_2.CH(NH_2).COOH$.
7. Cystine, $COOH.CH(NH_2).CH_2.S.S.CH_2.CH(NH_2).COOH$.
8. Tryptophane, $C_8H_6N.CH_2.CH(NH_2).COOH$.

It will be seen that all contain the amino-group —NH_2 and the carboxylic group —COOH. In addition, the following points may be noticed. Nos. 1 and 2 are examples of amino-derivatives of the simple fatty acids, nos. 3 and 4 are examples of amino-acids containing the aromatic group phenyl, C_6H_5—. Glutaminic acid, no. 5, is an example of one of these bodies containing two carboxylic groups and only one amino-group, lysine, no. 6, contains two amino-groups and only one carboxylic group, cystine, no. 7, contains the element sulphur, and tryptophane, no. 8, contains the indole group, C_8H_6N—.

Molecules of amino-acids are capable of condensing with one another. Take the case of two molecules of glycine as an example. The condensation can be represented by the equation:

$CH_2(NH_2).COOH + CH_2(NH_2).COOH$
  Glycine +   Glycine
    = $CH_2NH_2.CO.NH.CH_2.COOH + H_2O$
    =   Glycyl-glycine   + Water.

The glycyl-glycine is then again capable of condensing with another molecule of amino-acid, say another molecule of glycine, as shown below:

$CH_2.NH_2.COOH + CH_2.NH_2.CO.NH.CH_2.COOH$
  Glycine +   Glycyl-glycine
    = $CH_2.NH_2.CO.NH.CH_2.CO.NH.CH_2.COOH + H_2O$
    =   Di-glycyl-glycine   + Water.

The condensation need not, of course, take place between two molecules of the same amino-acids and probably does generally not do so, and the complicated molecules built up by the condensation of the different amino-acids are believed to form the bodies known as the proteins. Hydrolysis with concentrated acids effects the opposite reaction and the protein is broken down into its constituent amino-acids. The separation of all the amino-acids formed in a hydrolysis is a most difficult and tedious process but glutaminic acid happens to be fairly easily separated from the hydrolysis products of gluten and its separation is described in Expt. 1.

Note a. Not all the carboxylic groups in a protein molecule are condensed with amino-groups of other amino-acids and, of those not so condensed some have formed the corresponding amides, —$CONH_2$. The nitrogen in this form can, as has been seen in dealing with amides, be liberated as ammonia, by boiling with hydrolysing agents. In Expt. 2 this is done by boiling with strong hydrochloric acid, when the nitrogen in the amide groups is liberated and forms ammonium chloride by combination with the acid. This can then be broken up by the addition of an alkali and the ammonia distilled off and estimated.

XIX

ENZYME ACTION

1. Ptyalin. Warm some distilled water in a clean beaker to about 40° C. Rinse out the mouth with a portion of this and throw this liquid away. Then take about 20 c.c. of the warm water into the mouth, move it about as much as possible with the tongue and keep it in the mouth for about a minute. Transfer the water to a clean beaker and repeat the process once or twice. Well mix the solutions so obtained and filter. Preserve this filtrate. Into a test-tube place 5 c.c. of 1 per cent. starch paste and add to this 5 c.c. of the filtered diluted saliva obtained above. Mix well and place in a water bath which is maintained at about 38° C. Place a series of drops of dilute iodine solution on a white porcelain dish and to one of these transfer, from time to time, a drop of the starch mixture from the test-tube. Notice that the dark blue colour at first produced with the iodine changes, in successive tests, through various shades of red until only a reddish-yellow colour is obtained. When this stage is reached test a few c.c. of the liquid in the test-tube for a reducing sugar by boiling with Fehling's solution. (See note a.)

2. Repeat the above experiment but this time boil the filtered dilute saliva before adding it to the starch solution. Note that in this case no change of starch to sugar takes place. (See note b.)

3. Pepsin. Place a small piece of fresh washed fibrin or finely divided coagulated egg-white in each of four test-tubes. To the four test-tubes add the following:

(1) 5 c.c. of 1 per cent. pepsin solution and 5 c.c. 0·2 per cent. HCl.

(2) 5 c.c. of 1 per cent. pepsin solution and 5 c.c. of water.

(3) 5 c.c. of water and 5 c.c. of 0·2 per cent. HCl.

(4) 5 c.c. of the pepsin solution that has been previously boiled and 5 c.c. of 0·2 per cent. HCl.

Place the four test-tubes in a water bath at about 38° C. for about half an hour. Note that in (1) the fibrin swells up, becomes transparent and finally dissolves, in (2) the fibrin is unaltered, in (3) the fibrin swells up but does not dissolve and in (4) the result is the same as in (3). (See note c.)

4. Pancreatic ferments. Trypsin and Erepsin. Place a piece of freshly washed fibrin or finely divided coagulated egg-white in each

of four test-tubes. To the four test-tubes then add the following reagents:

(1) 5 c.c. of trypsin solution and 5 c.c. of 0·5 per cent. Na_2CO_3 solution.

(2) 5 c.c. of trypsin solution and 5 c.c. of water.

(3) 5 c.c. of trypsin solution and 5 c.c. of 0·4 per cent. HCl.

(4) 5 c.c. of trypsin solution previously boiled and 5 c.c. of 0·5 per cent. Na_2CO_3 solution.

Place the four test-tubes in a water bath at 38° C. and leave for some time. Note that only in the case of (1) will the fibrin dissolve. In (3) the fibrin will swell but will not dissolve, while in (2) and (4) no change will take place. (See note d.)

5. Look up the results obtained by fermenting sugar with yeast in Chap. IV. (See note e.)

NOTES

General note. An enzyme is a body which is capable of enormously increasing the rate at which some chemical reaction takes place. Before the addition of the enzyme to the reaction solution, the chemical reaction under consideration is probably proceeding so slowly that it is usual to consider that no reaction is taking place at all while the addition of the enzyme so increases the rapidity of the reaction, that it generally appears that the addition of this body has initiated the reaction. The chemical nature of the enzymes is not known since the methods by which enzyme preparations are obtained does not allow of their complete separation from other bodies. They are therefore only known through their action on other bodies and the action of various physical and chemical agents on them. The most important of their properties can be summarised shortly as follows:

(*a*) They are soluble in water but precipitated from solution by alcohol; (*b*) they are destroyed by heating their solutions to the boiling point of water, and generally even by heating to 65° C.; (*c*) they show their maximum activity at a temperature of about 40° C.; (*d*) they frequently require a definite small degree of acidity or alkalinity of the solution to exhibit their full activity; (*e*) very small quantities of certain foreign substances will frequently completely stop the action of an enzyme; (*f*) a small quantity of an enzyme is capable of converting an indefinite quantity of the substance on which it is acting into other substances; (*g*) the enzyme is itself unchanged at the end of the reaction; (*h*) the action of enzymes is specific, that is, an enzyme is capable of acting on only one or, at the most, a limited number of closely related compounds, and has no effect on other bodies. Some of the most important enzymes, the substances on which they act and the bodies formed are given below:

Enzyme	*Acting on*	*Forming*
Invertase	Cane Sugar	Dextrose and Levulose
Diastase	Starch	Maltose and Dextrin
Ptyalin	,,	,, ,,
Pepsin	Proteins	Albumoses and Peptones
Trypsin	,,	Polypeptides and Amino Acids
Erepsin	,,	,, ,, ,,
Zymase	Dextrose, Levulose, etc.	Alcohol, Carbon Dioxide.

Note a. The enzyme ptyalin converts the starch into maltose and dextrin and the reaction solution therefore gradually ceases to give the starch reaction with iodine.

Note b. The enzyme is destroyed by boiling and no reaction takes place.

N. & N.   8

Note c. The experiment shows that neither the enzyme pepsin alone nor hydrochloric acid alone is capable of destroying the protein fibrin but only the combination of the two. The destruction of the enzyme by boiling is also shown.

Note d. In this case it is shown that only when the pancreatic ferments act in alkaline solution does any reaction take place, neither enzyme nor alkali being effective alone. The destruction of the enzyme by boiling is also shown.

Note e. The yeast added supplies two ferments, the first, invertase, converts the cane sugar into dextrose and levulose while the second, zymase, converts the dextrose and levulose to alcohol and carbon dioxide.

XX

DETERMINATION OF THE CLASS TO WHICH AN ORGANIC SUBSTANCE BELONGS

As a first indication in the identification of an organic compound it is important to determine to what class it belongs.

The classes with which the student is concerned are as follows:

(a) (Hydrocarbons)	(h) Carbohydrates
(b) Alcohols	(i) Amides
(c) Aldehydes	(j) Amines
(d) Ketones	(k) Proteins
(e) Acids and their salts	(l) (Alkaloids)
(f) Esters	(m) (Purines, chiefly Uric Acid)
(g) Fats and Oils	(n) (Phenols).

The less important groups, from the point of view of this course are placed in brackets. The groups to some extent overlap, thus some carbohydrates are aldehydes and many oils and fats are esters but the above classification will be useful for identification purposes.

The following tests will put the student on the track of classifying any given compound. It must always be borne in mind however that the substance given for identification may be in solution in some solvent, generally water, and not in the form of the pure compound.

1. Note its general appearance and smell. Much help can be obtained from the smell, many of the aldehydes, ketones, some of the acids, the esters and the amines having very characteristic odours.

2. If a solid or pure liquid note its solubility or insolubility in water. If it is soluble in water it cannot belong to groups (a), (f), (g) or (l) above.

3. If it is soluble in water, or already in solution or a liquid, test the aqueous solution of it with litmus paper and note whether it is acid or alkaline in its reaction. If acid in reaction it will belong to group (e), if alkaline probably to group (j).

4. (a) Ignite a small quantity in a porcelain dish (if a solution has been given it must, of course, be evaporated to dryness first) and see whether there is any inorganic (non-combustible) residue. If there is, the substance is probably a salt of an organic acid (group (e)).

(b) Notice the odour on ignition. Many of the carbohydrates, especially the sugars, the oils and fats and some of the proteins have very characteristic odours on burning.

5. If the substance is insoluble in water, shake up with a little ether in a test-tube. If it dissolves, put a small quantity of the solution on white paper, allow the ether to evaporate and note whether an oily stain is left on the paper. If so the substance probably belongs to groups (*a*) and (*g*) or, less probably, to groups (*e*), (*f*) or (*j*).

6. To the substance, or to the solution, add caustic soda solution and boil. By the smell and with litmus paper note whether ammonia or an ammonia-like gas is evolved. If ammonia is evolved the substance is either the ammonium salt of an acid (group (*e*)) or an amide (*i*). If an alkaline gas is evolved, which is not ammonia, it is probably an amine (group (*j*)).

7. Heat the substance (or, if a solution is given, the solid obtained by evaporation) with soda lime in a dry test-tube. If ammonia or an ammonia-like gas is evolved the substance must be an ammonium salt of an acid (group (*e*)) or belong to groups (*i*), (*j*), (*k*), (*l*) or (*m*) and, if no ammonia was obtained in Expt. 6, it can only belong to groups (*j*), (*k*), (*l*) or (*m*).

8. It is a valuable confirmation of Expts. 6 and 7 to fuse the substance (or the solid obtained on evaporation, if a solution has been given) with a small piece of sodium and test for nitrogen and sulphur as described in Chap. I. If nitrogen is found the substance must belong to groups (*i*), (*j*), (*k*), (*l*) or (*m*) and if nitrogen and sulphur is found it probably belongs to group (*k*).

9. Dissolve the substance in water, add an excess of Fehling's solution and boil. If reduction takes place, with the production of red cuprous oxide, the substance belongs to groups (*c*) or (*h*).

10. Boil the substance or the solution with a small quantity of dilute hydrochloric acid for some minutes. Cool, neutralise the acid with caustic soda, add an excess of Fehling's solution and boil. If reduction of the Fehling's solution takes place, the substance belongs to groups (*c*) or (*h*) and, if no action on Fehling's solution was obtained in Expt. 9, it can only belong to (*h*).

11. Warm a solution of the substance with ammoniacal silver nitrate solution, as described in detail in Chap. VI. If reduction to metallic silver takes place the substance belongs to groups (*c*) or (*h*) or is tartaric or citric acid from group (*e*). If reduction only takes place on prolonged boiling the substance belongs to group (*d*).

12. Warm a solution of the substance with dilute acetic acid and a few drops of phenylhydrazine. Precipitation of a hydrazone or osazone places the substance in groups (*c*), (*d*) or (*h*).

13. If (*a*) the substance is soluble in dilute acids but is precipitated on adding caustic soda solution, or (*b*) if on boiling a dilute acid solution no characteristic smell is observed while on making the solution

alkaline with caustic soda and boiling a characteristic smell becomes noticeable, the body probably belongs to groups (*j*) or (*l*).

14. If the substance is soluble in dilute caustic soda solution by merely shaking, without warming, and is precipitated again by making the solution acid with a mineral acid, it probably belongs to groups (*e*) or (*n*) or is uric acid from group (*m*). If the substance only dissolves in dilute caustic soda solution on boiling for a few minutes and not in the cold on shaking, and a precipitate is or is not formed on acidifying with a mineral acid, it probably belongs to groups (*f*) or (*g*).

15. If (*a*) the substance is soluble in sodium carbonate solution with the liberation of carbon dioxide, or if (*b*) the substance in solution decomposes sodium carbonate with the liberation of carbon dioxide, the substance belongs to class (*e*).

16. Warm a mixture of the substance with a small quantity of alcohol and a little strong sulphuric acid. The fragrant smell of an ester indicates that the substance belongs to the acid group, group (*e*). (If the substance given is an aqueous solution this test will only succeed if the substance can be freed from water by evaporation or distillation.)

17. Repeat Expt. 16 but use a small quantity of glacial acetic acid instead of alcohol. A fragrant ester smell in this case, indicates that the substance is an alcohol, class (*b*). (The same warning about aqueous solutions applies.)

18. An oil which is not soluble on prolonged boiling with caustic soda solution and which is not soluble in dilute acids or acted upon on warming with concentrated acids, probably indicates a compound belonging to group (*a*).

As soon as the substance can be placed with any certainty in one or two of the groups mentioned above, the special tests for members of that group should be carried out on it, when not only its class, but, probably the individual member of that class will be identified.

It should seldom be necessary to carry out the whole of the above tests before a substance can be classified and frequently some information known with regard to the substance (its origin, purpose for which it is to be used, etc.) will limit the number of the groups into which the substance can fall.
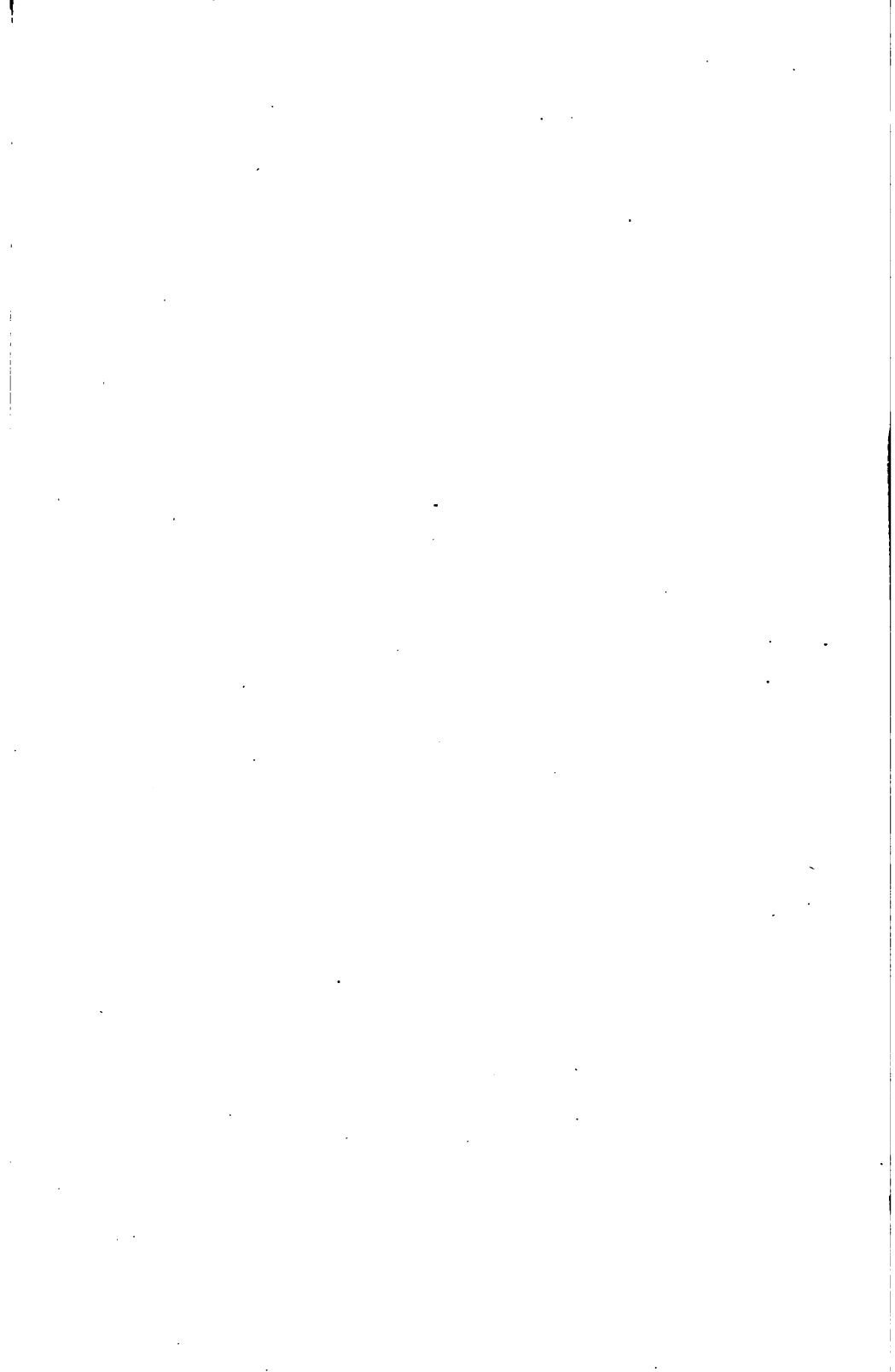
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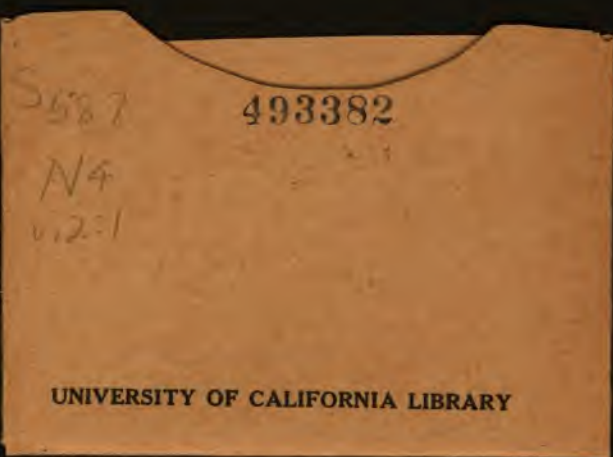DAY AND TO $1.00 ON THE SEVENTH DAY
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